Methylmercury determined by hplc– icpms in marine food and feed; in-house method validation and interlaboratory comparison

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Jana Pulkrabová, Monika Tomaniová, Michel Nielen and Jana Hajšlová
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INSTITUTE OF CHEMICAL TECHNOLOGY PRAGUE

RIKILT WAGENINGEN UR

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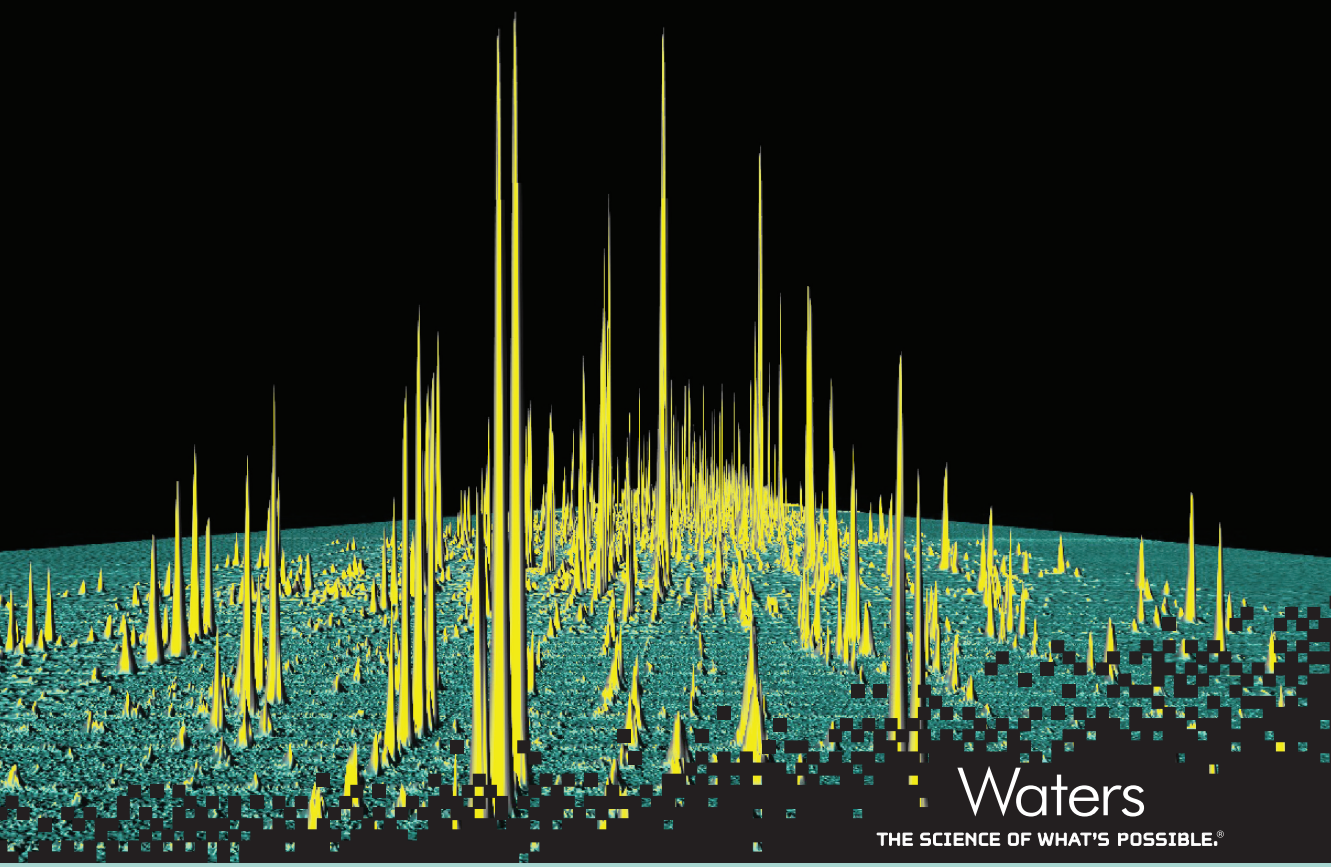

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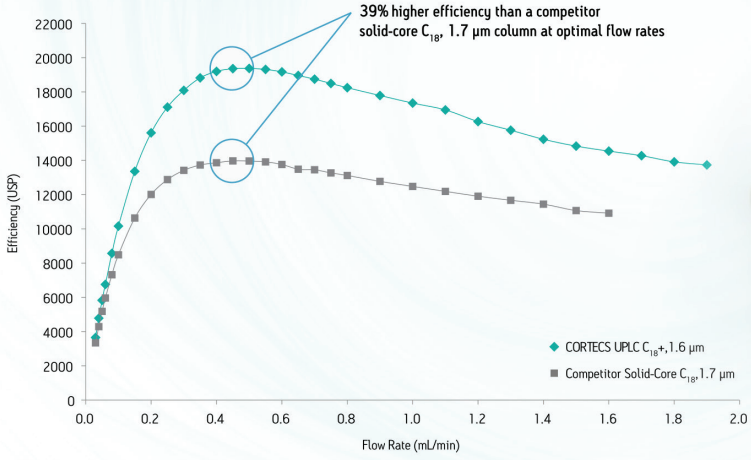

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SEMINARS
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SYMPOSIUM WORKSHOP, NOVEMBER 5, 2013 (9:00–13:00)

WORKSHOP ON OPPORTUNITIES TO WORK IN AND WITH A EUROPEAN SCIENTIFIC INSTITUTION

Registration for the workshop and welcome coffee from 9:00

9:30–9:40	**OPENING AND WELCOME** ***Dr. Elke Anklam**, Director of the EC–JRC–IRMM, Geel, Belgium* ***Dr. Franz Ulberth**, Head of the Standards for Food Bioscience Unit, EC–JRC–IRMM, Geel, Belgium* ***Prof. Dr. Jana Hajslova**, Head of Department of Food Analysis and Nutrition, Institute of Chemical Technology, Prague, Czech Republic* ***Dr. Nada Konickova**, Head of National Information Centre for European Research, Technology Centre ASCR, Prague, Czech Republic* ***Prof. Dr. Michel Nielen**, Principal Scientist at RIKILT–Wageningen University and Research Centre, Wageningen, The Netherlands* *Moderator of the workshop: **Franz Ulberth**, EC–JRC–IRMM, Geel, Belgium*
9:40–10:10	**NEW EU PROGRAM HORIZON 2020 IN SUPPORT OF FOOD RESEARCH AND INNOVATION** ***Patrik Kolar**, Head of Unit for Food, Health and Well-Being, EC–DG Research and Innovation, Brussels, Belgium*
10:10–10:40	**COLLABORATION OPPORTUNITIES OFFERED BY THE EUROPEAN COMMISSION'S JOINT RESEARCH CENTRE** ***Elke Anklam, Franz Ulberth**, EC–JRC–IRMM, Geel, Belgium*
10:40–11:00	**THE EUROPEAN INSTITUTE OF INNOVATION AND TECHNOLOGY (EIT) AND ITS KNOWLEDGE AND INNOVATION COMMUNITIES (KICS) – INTRODUCTION AND FUTURE PERSPECTIVES** ***Peter Olesen**, member of European Institute of Innovation and Technology (EIT) Governing Board*
11:00–11:30	Coffee break
11:30–11:50	**SUPPORT TO RESEARCHERS MOBILITY IN NEW EU PROGRAM HORIZON 2020** *Petra Perutkova, Technology centre ASCR, Prague, Czech Republic*
11:50–12:10	**PERSONAL EXPERIENCE OF A YOUNG SCIENTIST: MY RESEARCH ABROAD, FROM ICT PRAGUE TO JRC** ***Zuzana Zelinkova**, EC–JRC–IRMM, Geel, Belgium* **WORKING ABROAD BUT STILL WITH MOTHERS UNIVERSITY** ***Josep Rubert**, University of Valencia, Spain & Institute of Chemical Technology, Prague, Czech Republic*
12:10–12:30	**GET YOUR PhD IN FOOD SCIENCE IN THE CZECH REPUBLIC!** ***Jana Hajslova**, Institute of Chemical Technology, Prague, Czech Republic*
12:30–13:00	**QUESTIONS / ANSWERS** **CLOSING OF THE WORKSHOP**

LECTURERS

***Elke Anklam**, European Commission, Joint Research Centre (EC–JRC) – Institute for Reference Materials and Measurements (IRMM), Geel, Belgium*

Elke Anklam is a chemist by education with specialisation in food, organic and radiation chemistry. After having obtained her PhD from the University Hamburg, Germany, she worked in various European Research Institutions and was teaching as Professor in the Applied University of Fulda, Germany. Since 1991 she has been working in the European Commission's Joint Research Centre (JRC–EC). From 2006–2012 she was Director of the Institute for Health and Consumer Protection (JRC–IHCP) in Ispra, Italy and since January 2013 she is Director of the Institute for Reference Materials and Measurements (JRC–IRMM) in Geel, Belgium.

***Franz Ulberth**, European Commission, Joint Research Centre (EC–JRC) – Institute for Reference Materials and Measurements (IRMM), Geel, Belgium*

Franz Ulberth is the Head of the Standards for Food Bioscience Unit (until Dec 2012 known as Food Safety and Quality Unit) at the European Commission's Joint Research Centre – Institute for Reference Materials and Measurements (JRC–IRMM). Franz graduated (PhD) in "Food Science and Biotechnology" from the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna, Austria. After graduation he joined the Department of Food Science and Technology at the same university. Triggered by participation in research projects funded by the EC's Measurement & Testing Programme, his research interest soon included quality of analytical data and developing tools such as validated methods and reference materials for ensuring quality in the laboratory. He joined JRC–IRMM in 2002 as a programme co-ordinator for food and environmental reference materials at the IRMM. In 2007 Franz was nominated Head of the Standards for Food Bioscience Unit at the JRC-IRMM. He represents the Joint Research Centre in relevant Technical Committees of standards developing organisations such as the European Committee for Standardization, International Organization for Standardization, AOAC International and the Codex Alimentarius.

***Jana Hajslova**, Institute of Chemical Technology, Prague, Czech Republic*

Prof Jana Hajslova is the head of the Department of Food Analysis and Nutrition, Institute of Chemical Technology, Prague and its Laboratory of Food Quality and Safety. She is an expert in food chemistry and analysis and published widely more than 240 original papers on organic contaminants and chemical food safety. Her research team participated in many international and national projects at both research and project management levels, particularly in two projects funded by the EC 5th Framework programme, five projects funded by the EC 6th Framework programme, several COSTs and EEA grant. Recently and currently, she has been the ICTP team leader in charge of the scientific and technological aspects of the seven EC FP7 'Collaborative' and 'Coordination and support action' projects. Prof Hajslova participates in many international research activities and under her supervision close collaboration with many world-renowned institutions, such as WHO, FAO, USDA and the European Commission's Joint Research Centre has been established. She is also the Czech Republic delegate to the Cooperation Work Programme: Food, Agriculture and Fisheries, Biotechnology. As the chairwomen, she had a key input in establishing a series of very reputable international symposia "Recent Advances in Food Analysis" in 2003–2013.

***Nada Konickova**, Technology centre ASCR, Prague, Czech Republic*

Nada Konickova is the head of National Information Centre for European Research, the National Contact Point for the FP7 thematic area BIO and NCP for Joint Research Centre EC. She has been involved in several FP7 projects including BioNet – network of NCPs in FP7 thematic priority Food, agriculture and biotechnology. Her previous working experience, including work at Food and Agriculture Organization of the UN in Rome, was focused mainly at information systems for food and agriculture. She graduated from the Czech University of Agriculture and later on finished post-graduate study of information systems and services at Charles University in Prague.

***Michel Nielen,** RIKILT, Wageningen University and Research Centre, Wageningen, The Netherlands*

Prof. Dr. Michel Nielen is principal scientist at RIKILT, scientific director of TI-COAST, the Dutch public-private partnership on Analytical Science & Technology, and is professor of analytical chemistry at Wageningen University. He obtained his doctorate in analytical chemistry at the Free University of Amsterdam. He is co-founder and co-chairman of the symposium series on Recent Advances in Food Analysis (RAFA). He is (co-)author of more than 160 peer-reviewed publications.

***Patrik Kolar**, European Commission, EC–DG Research and Innovation, Head of Unit for Food, Health and Well-being, Brussels, Belgium*

Patrik Kolar obtained his PhD in chemistry at the University of Ljubljana, Slovenia in 1995. During 1997–1998 he worked as a PostDoc research fellow on the asymmetric catalysis involving proteinogenic amino acids at the University of Karlsruhe in Germany. Since 2000 he lives in Brussels, Belgium, where he initially worked as Research Advisor in the NGO Sector, and later as a Research Counsellor of the Slovenian Permanent Representation to the EU (2004–2007). He joined the European Commission in 2007 as the Head of Unit for Genomics and Systems Biology (2004–2010). He led the Personalised Medicine Unit from 2010 to 2013 when he joined the Directorate for Biotechnologies, Agriculture and Food as the Head of Unit for "Food, Health and Wellbeing". His Unit is responsible for designing policies and implementation of related research and innovation actions within the EU 7th Framework Programme (FP7) and the forthcoming European Framework Programme for Research and Innovation – the Horizon 2020. His Unit strongly collaborates with external stakeholders, including the European Technology Platform "Food for Life" and the Joint Programming Initiative on "Healthy Diets – Healthy Lives" (HDHL).

***Peter Olesen**, The European Institute of Innovation and Technology (EIT) Governing Board*

Peter Olesen is Chair of the Board of the Danish Council for Strategic Research. He spent 25 years as Research Director, VP R&D and Executive VP/CSO in four major international food and food ingredients companies: Danisco A/S (DK) 1983–93; Sandoz Seeds Ltd. (France) 1994–95; CPKelco/Hercules Inc. (DK & US) 1996–2000; Chr.Hansen A/S (DK) 2000–2008. He has held 3 appointments as affiliated professor at the University of Copenhagen: Plant Sciences 1986–91 and 1993–98; Functional Foods 2008–13. In addition, he was employed as part-time MSO professor at the Department of Plant Biology and Biotechnology at LIFE, University of Copenhagen 2009–11. Today, he is manager of his own company, ActiFoods, that offers counselling and knowledge sharing to companies and research institutions within food and health.

***Petra Perutkova**, Technology centre ASCR, NCP for specific programmes People and Ideas of FP7*

Petra Perutková is an expert in the relevant EC Programme Committees and member of the working group for human resources of the Ministry of Education, Youth and Sports of the Czech Republic. She has gained practical experience with EU framework programmes when coordination the FP7 project EuroNanoForum 2009 (http://www.euronanoforum2009.eu).

***Zuzana Zelinkova**, European Commission, Joint Research Centre (EC–JRC) – Institute for Reference Materials and Measurements (IRMM), Geel, Belgium*

Zuzana Zelinkova, Ph.D. is working as a post-doctoral research scientist at the European Commission's Joint Research Centre, Institute for Reference Materials and Measurements (JRC–IRMM) in Geel, Belgium. She graduated at the Institute of Chemical Technology Prague (ICT Prague), Department of Food Chemistry and Analysis (currently Department of Food Analysis and Nutrition). Her scientific work focuses mainly on food processing contaminants and their determination and validation of analysis method based on gas chromatography with mass spectrometry. Knowledge and professional skills gained during the studies and working at ICT Prague allowed her to get the postdoc position at the Standards for Food Bioscience Unit of JRC–IRMM. She is working in the European Reference Laboratory for Polycyclic Aromatic Hydrocarbons (PAHs) which is responsible for supporting national laboratories across the EU Member States, organisation of proficiency testing and assisting with the development and validation of analytical methods to harmonise official controls and help with further development of PAHs analysis.

***Josep Rubert**, University of Valencia, Spain & Institute of Chemical Technology, Prague, Czech Republic*

Dr. Josep Rubert has developed his doctoral Thesis in the Department of Preventive Medicine and Health Public, Food Science and Forensic Medicine, Faculty of Pharmacy, Universitat de Valencia (Spain). Over this period, Dr. Rubert was involved in development and optimization of analytical methods using LC–MS, analyzing mycotoxins in foodstuff and biological samples. During his doctoral thesis, Dr. Rubert has conducted two research stage visits. The first experience was carried out at the Cork Institute of Technology, CIT, Cork (Ireland) under the supervision of Dr. Kevin J. James in laboratory PROTEOBIO, where he had the opportunity to work, and for the first time, using UHPLC–HRMS. Secondly, Dr. Rubert was accepted to be involved in a research at Institute of Chemical Technology, ICT Prague, under the supervision of Prof. Jana Hajslova. This research was focused on the comparison of efficiency and efficacy of different extraction procedures for analyzing mycotoxins using UHPLC–HRMS. His doctoral thesis was defended in May 2012 at the Faculty of Pharmacy at Universitat de Valencia (Spain). During this time, 17 scientific papers were

published and national and international conferences were attended. Actually, Dr. Rubert works at the Institute of Chemical Technology of Prague, Department of Food Analysis and Nutrition, at a Postdoctoral position. He is involved in metabolomics fingerprinting/profiling as a tool for food authentication, fraud detection and new approaches.

HORIZON 2020 – NEW EU FRAMEWORK PROGRAMME SUPPORTING FOOD RESEARCH AND INNOVATION

***Patrik Kolar**, Unit E.3: Food, Health, Well-being, Directorate E: Biotechnologies, Agriculture, Food, Directorate General for Research and Innovation, European Commission, Patrik.KOLAR@ec.europa.eu*

'Horizon 2020' (H2020) is the new European Framework programme for research and innovation (R&I) covering the period 2014–2020, with an overall budget of approximately €72 billion. As a core element of the 'Europe 2020 Strategy' the Innovation Union and the European Research Area, H2020 has been designed to respond to the economic crisis by investing in future jobs and growth, addressing people's concerns about their livelihoods, safety and environment, and strengthening the EU's global position in research, innovation and technology. H2020 integrates all EU-supported activities on R&I into a single programme to allow support to projects "from the laboratory to commercial exploitation".

Activities under Horizon 2020 are focused on three priorities: excellent science, industrial leadership and societal challenges. Most of the activities related to food R&I are foreseen within the Societal Challenge (SC) 'Food security, sustainable agriculture and forestry, marine and maritime and inland water research and the bioeconomy', which has a dedicated budget of €3,851 million.

Under this challenge, a number of research areas will be addressed including, in the food area: informed consumer choices, healthy and safe diets and a competitive agri-food industry.

High level research, innovative models and new methodologies will be applied to investigate the preferences, needs, behaviour, and lifestyle of consumers, to promote communication between consumers and the food chain research community and its operators, to study the impact on diet related diseases and to identify sustainable eating behaviours.

Healthy diets will be explored through research that will focus on providing dietary solutions and innovations leading to improvements in health and wellbeing. The links between diet, ageing, chronic diseases and disorders, and dietary patterns will also be investigated together with the nutritional needs and the impact of food on physical and mental performance.

Safe diets will be achieved by addressing the need to monitor, control and trace chemical and biological contamination throughout the food, feed and drinking water supply chain and new risk assessment strategies will be explored and regulatory frameworks will be examined. New methods, tools and mechanisms will be developed that will reinforce the integrated approach on food safety. Finally, food safety standards will be created or improved in order to contribute to the safety of international trade.

The competitiveness of the agri-food industry will be enhanced through R&I that will include all stages of the production chain (from design to the market) addressing such critical issues as affordability, quality, traceability and logistics together with accompanying social, environmental and economic concerns.

At the time of writing, the proposal for H2020 is awaiting formal adoption by the Council and the European Parliament. The first calls for proposals are foreseen for publication in December 2013.

SYMPOSIUM WORKSHOP, NOVEMBER 5, 2013 (9:00–13:00)

WORKSHOP ON INFRARED SPECTROSCOPY, RAMAN SPECTROSCOPY AND CHEMOMETRICS FOR MONITORING OF FOOD AND FEED PRODUCTS, LAB-TO-THE-SAMPLE

Chairs: Vincent Baeten & Juan-Antonio Fernández Pierna, *Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium*

8:30–9:00	Registration for the workshop
9:00–10:00	**BASICS OF INFRARED AND RAMAN SPECTROSCOPY** ***Vincent Baeten****, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*
10:00–11:00	**BASICS OF CHEMOMETRICS** ***Juan-Antonio Fernández Pierna****, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*
11:00–11:30	Coffee break
11:30–11:50	**CHALLENGES OF VALIDATION OF SPECTROSCOPIC METHODS: THE EXAMPLE OF MEAT AND BONE MEAL (MBM) DETECTION** ***Christoph von Holst****, EC–JRC–Institute for Reference Materials and Measurements (IRMM), Geel, Belgium*
11:50–12:10	**NEW TOOLS FOR THE DAIRY SECTOR BASED ON MIR AND NIR SPECTROSCOPY** ***Clément Grelet****, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*
12:10–12:30	**APPLICATION OF FT-IR SPECTROSCOPY FOR AUTHENTICATION OF DISTILLERS DRIED GRAINS AND SOLUBLES (DDGS)** ***Thorben Nietner****, Federal Institute for Risk Assessment, Berlin, Germany*
12:30–12:50	**TRANSFER OF METHODOLOGY FROM LAB TO INDUSTRY FOR THE DETECTION OF ERGOT** ***Philippe Vermeulen****, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*
12:50–13:00	**DISCUSSION & CONCLUSION OF THE WORKSHOP** ***Vincent Baeten****, Walloon Agricultural Research Centre (CRA–W), Gembloux, Belgium*

Vibrational spectroscopy, as Near infrared (NIR) or Raman, is the most widely used non-destructive technology in the food and feed industries for the daily determination and quantification of qualitative parameters of the materials. The high throughput of the method, the capacity to determine in one single analysis a panoply of parameters, the possibility to build a network of spectrometers together with its potential use both on-line and at-line in a production plant made this technique even more attractive. These techniques provide real-time analyses with an increased sample throughput. Moreover, more recent areas as hyperspectral imaging allow collection of spectroscopic images at different levels from single kernel or particle levels to satellite. This is of great interest for laboratories that control feed compound or cereals. Other decisive advantages of spectroscopic methods are the ability to determine simultaneously different parameters and criteria, no use of reagents and reduced sample preparation.
The combination of these techniques with appropriate data treatment or chemometric tools should help to solve the deep and rapid changes that the agro-food sector is facing with increasing consumer concerns about food and feed safety and quality issues. These concerns arise in part from previous safety crises (e.g. dioxin, BSE, melamine) and in part from the health impact of food and feed. The main outcome of these consumer demands is an increased need for appropriate techniques and methods to help producers, retailers and processors to control and to track their products. Infrared and Raman spectroscopy combined with chemometric should allow to build strategies that can be applied to check (on-line, at-line and at the laboratory level) the quality of food and feed materials, to detect non conformity and subsequently to identify targeted or untargeted adulterants and contaminants among others.

WEDNESDAY, NOVEMBER 6, 2013 (9:00–10:30)

EFSA SEMINAR ON RISK ASSESSMENT ON CONTAMINANTS IN FOOD AND FEED

Chair: Mari Eskola, *Acting Head of Unit, European Food Safety Authority, Unit on Contaminants, Parma, Italy*

9:00–9:15	**EFSA CONTAM PANEL: HOW IT CARRIES OUT RISK ASSESSMENTS ON CONTAMINANTS** ***Mari Eskola****, EFSA CONTAM Unit, Parma, Italy*
9:15–9:30	**COLLATION OF CHEMICAL OCCURRENCE DATA FOR EXPOSURE ASSESSMENT** ***Fanny Heraud****, EFSA DCM Unit, Parma, Italy*
9:30–9:45	**EFSA CONTAM PANEL RISK ASSESSMENTS OF HEAVY METALS – THE EXAMPLE OF MERCURY AND METHYLMERCURY IN FOOD** ***Katleen Baert****, EFSA CONTAM Unit, Parma, Italy*
9:45–10:00	**EFSA CONTAM PANEL RISK ASSESSMENTS OF BROMINATED FLAME RETARDANTS (BFRs) IN FOOD** ***Luisa Ramos Bordajandi****, EFSA CONTAM Unit, Parma, Italy*
10:00–10:15	**RISK ASSESSMENTS ON MYCOTOXINS IN FOOD AND FEED – SCIENTIFIC OPINIONS OF THE EFSA CONTAM PANEL** ***Mari Eskola****, EFSA CONTAM Unit, Parma, Italy*
10:15–10:30	**Questions and discussion**

EFSA sponsors coffee break that follows after the seminar.

You are welcome to attend the EFSA seminar and continue with the discussions during the coffee break sponsored by EFSA.

WEDNESDAY, NOVEMBER 6, 2013 (16:00–18:00)

INTERACTIVE SEMINAR ON SAMPLE-PREP APPROACHES, SEPARATION TECHNIQUES AND APPLICATION OF MASS SPECTROMETRY IN FOOD QUALITY / SAFETY CONTROL: STEP BY STEP STRATEGIES FOR FAST DEVELOPMENT OF ANALYTICAL METHOD

Sponsored by

Moderators:

Dr. Katerina Mastovska *(Covance Laboratories, Greenfield, IN, USA)*

Dr. Hans Mol *(RIKILT Wageningen UR, Wageningen, the Netherlands)*

Dr. Lukas Vaclavik *(Institute of Chemical Technology, Prague, Czech Republic & Food and Drug Administration, USA)*

ANNOTATION

This educative seminar is intended for young scientists but all other RAFA attendees are also welcome! It will provide interactive demonstration of general approaches to fast development and troubleshooting of analytical methods for food quality and safety control. The moderators will introduce several case studies with various conceivable scenarios for each step in the method development (including both sample preparation and instrumental analysis) and/or for each troubleshooting problem. Each time, the attendees will identify the most suitable solution using an anonymous electronic voting system, followed by an interactive discussion about each presented option. In the end of the seminar, groups of participants will compete against each other by proposing optimal solutions for particular analytical problems. The best ideas will be awarded by special prizes!

We encourage you to attend this informal and interactive seminar, which was very well received at RAFA in 2011. Come to join the discussion, outline your vision, learn something new and have some fun!

All attendees on the board through your voting device!

Please note the capacity of the seminar is limited.

THURSDAY, NOVEMBER 7, 2013 (9:00–12:30)

2nd EUROPEAN AMS WORKSHOP ON AMBIENT MASS SPECTROMETRY IN FOOD AND NATURAL PRODUCTS

Associated event

Ambient MS training platform

www.qsaffe.eu

Chairs:

***Christian Klampfl**, Johannes Kepler University, Linz, Austria*

***Facundo Fernandez**, Georgia Institute of Technology, Atlanta, GA, USA*

Workshop is dedicated to all laboratories concerned with food / natural products analysis. The mission of this workshop is to introduce challenging innovations in ambient mass spectrometry-based measurements and, last but not least, bring together all current and future users.

9:00–9:30	**AMBIENT MASS SPECTROMETRY: A TUTORIAL** ***Facundo Fernandez**, Georgia Institute of Technology, Atlanta, GA, USA*
9:30–9:50	**ACCURATE MASS FRAGMENT LIBRARY FOR RAPID SCREENING FOR PESTICIDES ON THE SURFACE OF IMPORTED PRODUCE USING AMBIENT PRESSURE DESORPTION IONIZATION WITH HIGH-RESOLUTION MASS SPECTROMETRY** ***Sara Kern**, United States Food and Drug Administration, Cincinnati, OH, USA*
9:50–10:10	**DIRECT FRUIT PEEL ANALYSIS BY DART–ORBITRAP–MSN** ***Marinella Farré**, Department of Environmental Chemistry, IDAEA–CSIC, Barcelona, Spain*
10:10–10:30	**RAPID WINE PROFILING STRATEGIES: EXPLORING AN AMBIENT IONIZATION METABOLOMICS APPROACH COUPLED WITH EXTRACTION TECHNIQUES** ***Elizabeth Crawford**, Institute of Chemical Technology, Prague, Czech Republic*
10:30–11:00	***Coffee Break***
11:00–11:30	**NOVEL MS TECHNIQUES FOR THE ANALYSIS OF FOOD AND FOOD CONTACT MATERIALS** ***Christian Klampfl**, Johannes Kepler University, Linz, Austria*
11:30–11:50	**RECENT ADVANCES AND CHALLENGES OF AMBIENT IONIZATION MASS SPECTROMETRY IN FOOD ANALYSIS** ***Michel Nielen**, Wageningen University, Wageningen, The Netherlands*
11:50–12:10	**LESA ANALYSIS OF BACTERIAL SURFACE: FROM MICROBIAL COMMUNICATION TO FOOD SAFETY** ***Ales Svatos**, Mass spectrometry/Proteomics Research Group, Max Planck Institute, Jena, Germany*
12:10–12:30	**DART–MS: AN OVERVIEW OF CONCEIVABLE APPLICATIONS IN FOOD ANALYSIS AND AUTHENTICATION** ***Jana Hajslova**, Institute of Chemical Technology, Prague, Czech Republic*

THURSDAY, NOVEMBER 7, 2013 (16:00–18:00)

SEMINAR ON FOOD SAFETY ISSUES BEYOND THE EU

***Chair: James Lindsay, Ph.D, FAAM, Professor**, National Program Leader Food Safety, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), Beltsville, MD, USA*

16:00–16:20 **PREHARVEST MYCOTOXIN CONTAMINATION OF AGRICULTURAL COMMODITIES: CURRENT ISSUES ON DETECTION AND CONTROL**
***Deepak Bhatnagar**, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), New Orleans, MD, USA*

16:20–16:40 **"MASKED" MYCOTOXIN DETECTION: WHAT IS A POOR CHEMIST TO DO?**
***Chris Maragos**, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), Peoria, IL, USA*

16:40–17:00 **QUANTITATION OF AFLATOXINS FROM CORN AND OTHER FOOD RELATED MATERIALS BY DIRECT ANALYSIS IN REAL TIME – MASS SPECTROMETRY (DART–MS)**
***Mark Busman**, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), Peoria, IL, USA*

17:00–17:20 **HEXABROMOCYCLODODECANE AS A PROTOTYPE OF THE ANALYTICAL CHALLENGES OF EMERGING FLAME RETARDANTS IN FOODS**
***Heldur Hakk**, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), Fargo, ND, USA*

17:20–17:40 **DIOXIN, FURAN, PCB, AND PBDE LEVELS IN U.S. FOODS: SURVEY TRENDS AND CONSUMER EXPOSURE**
***Sara Lupton**, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), Fargo, ND, USA*

17:40–18:00 **MULTI-CLASS, MULTI-RESIDUE ANALYSIS OF ENVIRONMENTAL CONTAMINANTS AND PESTICIDES IN FISH USING FAST, LOW-PRESSURE GAS CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY**
***Yelena Sapozhnikova**, U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS), Wyndmoor, PA, USA*

THURSDAY, NOVEMBER 7, 2013 (16:00–18:00)

TUTORIAL ON TOOLS FOR MASS SPECTROMETRY-BASED METABOLOMIC DATA PROCESSING AND ANALYSIS

***Moderator: Dr. Tomas Cajka**, Metabolomics Fiehn Lab, Genome Center, University of California, Davis, USA*

The metabolomics approach, aiming at global analysis of numerous targeted or non-targeted low molecular compounds (metabolites) in a biological sample, has recently found its application in diverse research areas including food quality and safety, and authenticity. A rapid growth of metabolomics has been enabled by substantial advances in analytical techniques such as mass spectrometry (MS) coupled to liquid chromatography (LC) or gas chromatography (GC), and nuclear magnetic resonance (NMR), all the techniques facilitating analysis of a wide range of metabolites with diverse physicochemical properties and occurring at different concentration levels. To process and interpret the complex data obtained within metabolomic-based studies, advanced software algorithms of data handling are needed, consisting of data processing, data pretreatment, and data analysis.

Data processing proceeds through multiple stages such as filtering, peak detection, deconvolution, alignment, and normalization. The need of powerful data-processing methods gave rise to numerous commercial as well as free tools implementing one or several steps of the data processing pipeline.

Data pretreatment represents another crucial step that can dramatically change the outcome of the data analysis. This procedure typically involves centering and scaling of the original data to eliminate unwanted systematic bias, while maintaining genuine differences in the examined datasets.

Data analysis involves the use of various chemometric tools. Unsupervised pattern recognition techniques (represented mainly by principal component analysis) are often the first step of the data analysis in order to detect patterns in the measured data. On the other hand, supervised pattern recognition techniques (*e.g.*, partial least-squares discriminant analysis, linear discriminant analysis) use the existing information about the class membership of samples to a given group (class or category) to classify a new “unknown” sample using its pattern of measurement. From this point of view, the outputs of metabolomic data analysis may differ depending on the purpose of investigation.

In most cases, there is also an interest in ***identification*** of the discriminating (bio)marker compounds. In this case, the use of a high-resolution instrument enabling to obtain both single MS and MS/MS accurate mass spectra is needed for reliable elemental formula estimation, which is typically followed by a database search. In this tutorial, a brief overview of the key steps involved in data handling and interpretation in mass spectrometry-based metabolomics (covering GC–MS and LC–MS instrumental platforms) will be presented. Case studies and practical examples will be used to demonstrate these concepts.

VENDOR SEMINARS

VENDOR SEMINAR: NOVEMBER 6, 2013 (7:30–8:30)

FUNDAMENTALS OF SAMPLE PREPARATION METHOD DEVELOPMENT IN FOOD ANALYSIS

Fundamentals of Sample Preparation Method Development in Food Analysis

Andrea Gheduzzi

Routine analyses of food and environmental samples are often confounded by the extremely complex nature of the various sample matrices. The use of sample preparation techniques prior to gas or liquid chromatography is often essential to the success of an analytical method as they can both remove potential interferences and also greatly increase sensitivity. In this seminar, we will provide an overview of some of the most commonly-used sample preparation techniques for food and environmental samples, and offer some tips and tricks for maximizing their effectiveness.

VENDOR SEMINAR: NOVEMBER 6, 2013 (13:15–14:15)

ENHANCING THE VALUE OF DIRECT ANALYSIS IN REAL TIME (DART)–MS BY LEVERAGING SORBENT TECHNOLOGIES

Deploying DART in Analytical Workflows

***Facundo Fernandez**, Georgia Institute of Technology, Atlanta, Georgia, USA*

DART is the most popular of the plasma-based ambient mass spectrometry ionization techniques, allowing for direct analysis of a variety of solid, liquid and gaseous samples without sample preparation. It can be used both in quantitative and qualitative experiments, and can provide the information necessary for identifying unknowns when coupled to high resolution accurate mass MS instruments. In this presentation we will provide examples on how DART is being used in a multi-technique tiered approach to detecting falsified medicines. Examples will include counterfeit contraceptives, antimalarials and antimicrobials. Tips and tricks necessary for optimum DART operation will be provided, and a comparison with other ambient techniques, such as DESI, discussed.

Low-level Pesticide Screening and Monitoring Wine Spoilage: Rapid Sample Concentration for Quantitative Analysis by DART Mass Spectrometry

***Elizabeth Crawford*[1,2], Paola Domizio[3,4], Brian Musselman[1], C. M. Lucy Joseph[3], Linda F. Bisson[3], Bart C. Weimer[5] and Richard Jeannotte[5,6]**

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[2] Department of Food Analysis and Nutrition, Institute of Chemical Technology, Prague, Technická 3, 16628 Prague 6, Czech Republic

[3] Department of Viticulture & Enology, University of California-Davis, Davis, CA 95616, USA

[4] Dipartimento di Gestione Sistemi Agrari, Alimentari e Forestali (GESAAF), Università degli Studi di Firenze, 50144 Firenze, Italy

[5] Department of Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA

[6] Facultad de Ciencias, Universidad de Tarapacá, Arica, Chile

Pesticide screening and food spoilage monitoring are two important areas in food safety analysis. Rapid and sensitive screening methods ensure timely identification of emerging issues and increased safety for the public, as well as improved production conditions for manufacturers. A major source of wine spoilage worldwide is caused by the yeast Brettanomyces bruxellensis, which is responsible for significant economic loss in the wine and beer industries. Wines exhibiting a “Brett” character generally include medicinal and barnyard odors that are attributed to two volatile phenols, 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG). When 4-EP and 4-EG are at levels less than 400 µg/L they contribute positively to the sensory complexity of the wine giving it a spicy, smoky and leather aroma. However, when levels are greater than 620 µg/L, the “Brett” character may be too strong and the wine contains off-flavor notes and is often no longer marketable.

A Direct Analysis in Real Time (DART) ambient ionization source was coupled to a high resolution accurate mass (HRAM) quadrupole mass spectrometer for full scan pesticide screening and quantitative targeted MS/MS analysis of 4-EP and 4-EG markers in wine spoilage. Both analyses employed a rapid unattended sample concentration step using Twister stir bar sorptive extraction (SBSE) prior to ambient desorption ionization. The detection limits were in the very low ppb range for the targeted wine analyses and demonstrated an increased detection for a range of pesticides. Sample analysis time using the DART ionization approach was 3 minutes or less per sample to thermally desorb the analytes directly from the Twister sorptive stir bar surface. Rapid monitoring methods enhance consumer safety, protect the integrity of the product and allow the manufacturers to take control to prevent economic losses.

Leveraging Solid Phase Microextraction devices for removal of carbohydrates as a means of improving the quality of DART–MS results

***Brian Musselman**, Robert Goguen and Joseph Lapointe*, *IonSense, Inc., Saugus, Massachusetts, USA*

Determination of composition and quality of herbal supplements are time consuming due to the nature of the active components and complexity of the sample matrix. In our work with DART–MS we observed that the presence of carbohydrates limits the utility of the method by creating a matrix effect where significant ion current is detected as the protonated sugars. In order to reduce the effect of those sugars on the results we have incorporate solid phase micro-extraction (SPME) of supplement extracts as a simple sample prep method for eliminating the sugar matrix effect. Flexible and rugged sorbent coated wire solid phase extraction devices (Supelco LC–Probe devices) have been evaluated for use in this application. The sorbent coated wires were utilized for both sample extraction and as a support for direct surface desorption ionization facilitating rapid analysis.

Results using this new direct analysis from sorbent coated wire method with supplements and foodstuffs will be described. Post–SPME Derivatization of samples to permit detection of thermally labile compounds such as polyols will also be discussed with results from recent analysis of teas and supplements.

VENDOR SEMINAR: NOVEMBER 6, 2013 (13:15–14:15)

BRUKER DALTONICS:

MASS SPECTROMETRIC STRATEGIES FOR THE ACCURATE QUANTITATION AND SCREENING OF RESIDUES IN FOODS

Routine Quantitation of Pesticides by GC/MS/MS and LC/MS/MS

Kefei Wang, *Bruker Daltonics*

With approximately 1,000 pesticides being used globally, the requirement to monitor their correct use in accordance to international guidelines is a challenging task. In this presentation we show how both GC and LC -triple quadrupole mass spectrometry systems have been deployed to achieve rapid, targeted multi-residue quantitative analyses with industry leading sensitivity, reproducibility and ruggedness. Examples of real life analysis and methods development will be discussed.

The Next Generation of Ultra-High Resolution Q–TOF Platforms for Rapid Screening of Unknown Contaminants in Food

Carsten Baessmann*, Bruker Daltonics*

Targeted, rapid screening of pesticide residues in foods is becoming more commonplace in food testing laboratories and both nominal mass and high resolution, accurate mass LC–MS/MS systems have been successfully deployed for this purpose. In this presentation we describe how state of the art, Ultra High Resolution (UHR) Q–TOF technology and the Bruker PesticideScreener™ solution is used to screen for hundreds of pesticide residues in complex food matrices. The continuous acquisition of both MS and All Ion-CID spectral data throughout the entire chromatographic process coupled with intelligent data processing tools ensures the effective reduction of false positives and negatives.

VENDOR SEMINAR: NOVEMBER 6, 2013 (13:15–14:15)

NEW LEVELS OF CONFIDENCE AND PRODUCTIVITY IN THE SCREENING AND QUANTITATION OF RESIDUES AND CONTAMINANTS BY UHPLC/Q–TOF/MS

Agilent Technologies

New levels of confidence and productivity in the screening and quantitation of residues and contaminants by UHPLC/Q–TOF/MS

***Thomas Glauner*[1], *Guenther Kempe*[2], *Elisabeth Varga*[3], *Michael Sulyok*[3], *Rainer Schuhmacher*[3], *Rudolf Krska*[3] *and Franz Berthiller*[3]**

[1] *Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn, Germany*

[2] *LUA Saxony, Chemnitz, Germany*

[3] *University of Natural Resources and Life Sciences, Vienna (BOKU), Dept. for Agrobiotechnology (IFA-Tulln), Christian Doppler Laboratory for Mycotoxin Metabolism and Center for Analytical Chemistry, Tulln, Austria*

There is an ever increasing number of residues and contaminants which need to be tested in our daily food. The range of compounds includes pesticides, mycotoxins, packaging contaminants, and many others. For many control laboratories it would be extremely interesting to expand their scope of analysis to potentially include all these compound classes in one screening method. With modern Q–TOF LC/MS instruments, allowing the analysis of most pesticides and mycotoxins much below the regulatory limits, this is within the realms of possibility, not least since QuEChERS has proven its potential as generic sample preparation technique. Qualitative screening with Q–TOF analyzers in combination with comprehensive databases and libraries does not always require having expensive standards for each contaminant on hand. However, in complex matrices such as QuEChERS extracts of food, the challenge remains not only to find the targeted contaminants, but to successfully rule out potential false positives, and at the same time to be as productive as with LC triple quadrupole instruments.

In this presentation we show how this can be achieved when screening for pesticides and mycotoxins in various food matrices by applying Agilent's 1290 UHPLC system coupled to a Q–TOF LC/MS system operated in the All Ions MS/MS acquisition mode. The All Ions MS/MS allows for the CID fragmentation of compounds without precursor selection, enabling the accurate mass acquisition of molecular ions and fragments from the same compound. In combination with Agilent's unique offering of comprehensive databases and libraries and together with the industry leading MassHunter software, the All Ions MS/MS aids in the successful elimination of potential false positives. The workflow was successfully validated for the screening of pesticides in fruit and vegetables with a screening detection limit (SDL) below 5 µg/kg for most pesticides in all tested matrices. Further examples from the qualitative screening of mycotoxins in nuts and cereals will be shown, applying a new accurate mass database and library for mycotoxins and fungal metabolites.

The All Ions MS/MS workflow is perfectly integrated in the MassHunter software. Identification of contaminants is done on multiple levels using a unique co-elution score for the molecular ions and fragments, the comparison of area ratios, the accurate mass and isotope pattern matching of the molecular ions and the accurate masses of the fragments. Fast data review is aided by powerful graphical features of Batch- and Compounds-at-a-glance in combination of using all these ID criteria with MassHunter Quant outlier flagging.

VENDOR SEMINAR: NOVEMBER 6, 2013 (14:30–15:30)

INTRODUCING THE Rxi-PAH: A NEW OPTIMIZED GC COLUMN FOR PAH ANALYSES

Introducing the Rxi-PAH: A New Optimized GC Column for PAH Analyses

PAH analyses are performed regularly throughout the world for both food safety and environmental testing but challenges remain. The Rxi-PAH GC stationary phase was tailored specifically to meet these challenges. Even difficult priority compounds, such as the European Food Safety Authority (EFSA) PAH4, are easily separated and accurately quantified, results that cannot be achieved on typical GC columns.

An arylene modification and surface bonding of the stationary phase increase thermal stability and ruggedness so relatively nonvolatile, higher molecular weight PAHs can be analyzed routinely without interference from column bleed. For example, over thirty-five PAHs were analyzed in 33 minutes and included coronene and the dibenzo pyrenes eluting at high temperature with better detectability, peak height, than with other columns. In addition to this highly selective column, hydrogen carrier gas was explored with GC–TOFMS. PAHs are ideal for analysis by hydrogen carrier GC–MS because they form strong molecular ions and do not have problems with hydrogen reactivity. The fact that there is only one strong ion *m/z* signal helps overcome some sensitivity loss when using hydrogen in GC–MS.

Excellent column efficiency means that the column can be trimmed for maintenance purposes many times without losing critical PAH separations, including those that are part of environmental methods, as well as food safety testing. Column trimming from 60 to 45 meters was performed and resolution of critical pairs was maintained. Column ruggedness was tested with repeated injections of oyster and paprika QuEChERS extracts.

VENDOR SEMINAR: NOVEMBER 6, 2013 (14:30–15:30)

SHAQUE YOUR PESTICIDE ANALYSIS

ShaQue your pesticide analysis

Mark Tutty and Erika Felici

We will look at the importance of good sample preparation to the analytical process. Using data from a QuEChERS study, will demonstrate how mechanical equipment such as ShaQuer & GenoGrinder 2010 help improve consistency, throughout and pesticide recovery from a variety of samples.

The importance of using high quality standards and QuEChERS reagents will also be discussed.

VENDOR SEMINAR: NOVEMBER 6, 2013 (14:30–15:30)

GAIN CONFIDENCE IN YOUR RESULTS: EFFECTIVE APPLICATION OF THERMO SCIENTIFIC TECHNOLOGIES FOR SAFER FOOD

Thermo SCIENTIFIC

We will present recent developments and advances in analytical chemistry of emerging food contaminants and residues. The presented applications will cover from novel application of gas chromatography coupled to tandem mass spectrometry (GC–MS) for determination and confirmation of persistent pollutants like polychlorinated dioxins and dibenzofurans (PCDD/PCDF) in foods. The potential of novel high resolution and accurate mass spectrometry system for screening, quantitation and confirmation of growth promoters in urine will be described. Finally, advanced stable isotope ratio mass spectrometry (SIRMS) approach for assessment of authenticity and origin of foods will be presented.

Topics will be presented by guest speakers from leading scientific institutes across Europe and application specialists of Thermo Scientific.

Legacy and emerging POPs in food; analysis using GC–MS/MS

***Martin Rose**, Food and Environmental Research Agency, York, UK*

Measurement of dioxins in foods is probably the most challenging routine analysis carried out in order to ensure compliance with regulations. This has until now been undertaken by highly specialized laboratories equipped with high resolution mass spectrometers. These are sector instruments and require highly skilled operators in addition to a variety of services and facilities such as cooling water, 3 phase power supply etc.There are a variety of new and emerging contaminants and legacy contaminants that also pose potential dioxin-like health risks – brominated and mixed halogenated dioxins fall into the first of these categories and PCNs fall into the second. Recent advances in MSMS technology mean that this technique is a viable alternative to HRMS for regulatory monitoring and also for investigating non-regulated or near-regulated contaminants. This presentation will cover experiences of using the Thermo TSQ Quantum XLS Ultra as a tool for analysis of foods for a range of environmental contaminants.

Q-Exactive a versatile Mass-Spectrometer to detect growth promoters in urine?

***Marco Blokland**, RIKILT, Wageningen, Netherlands*

In Europe hormones are illegally used to enhance growth in cattle. Detection of abuse of growth promoters usually takes place through targeted MS analysis on a limited set of substances. Attempts to detect growth promotors in urine, using full-scan technologies, at residue levels in cattle urine has failed so far due to the low concentration of these compounds present in samples of urine. Results of the exploration of the Q-Exactive as a mass-spectrometer to detect growth promoters at residue levels in urine are presented.

Latest Developments in Isotope Ratio Mass Spectrometry: Authenticity control, Fraud and Forensics in Food

***Dirk Krumwiede**, Thermo Fisher Scientific, Bremen, Germany*

Isotope ratio mass spectrometry (IRMS) is a key technology for authenticity control and food testing. In a first step prepared food samples are applied to the mass spectrometer using a variety of different sample inlet systems including elemental analyzer, liquid and gas chromatography. The introduced sample is completely converted into simple gases (e.g. CO_2, H_2) by combustion or high temperature conversion devices prior to the final accurate determination of related isotope ratios ($^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{18}O/^{16}O$, $^{15}N/^{14}N$) in the sector field mass spectrometer. These highly precise measurements allow the detection of non-authentic food products or food fraud, like e.g. food supply chain adulteration. This presentation is aiming to give a brief introduction into IRMS, which is followed by some specific application examples for IRMS in food analysis. Finally some latest developments in the field of GC–IRMS will be presented including hyphenation and column connection technology.

VENDOR SEMINAR: NOVEMBER 6, 2013 (18:30–19:30)

THE CHANGING ROLE OF LC–MS/MS IN FOOD TESTING. A LOOK AT THE LATEST SOLUTIONS FOR INGREDIENT PROFILING AND CONTAMINANT DETECTION BY LC–MS/MS

The changing role of LC–MS/MS in food testing. A look at the latest solutions for ingredient profiling and contaminant detection by LC–MS/MS

Andre Schreiber, *with guest speaker from Phenomenex*

Food testing scientists work endlessly to monitor our food supply for hazardous chemicals and contaminants to ensure human and animal safety, and that often means testing hundreds of samples for hundreds of chemical compounds every day, and with a fast turnaround of results. This presentation will describe new innovations in LC–MS/MS hardware and workflows that will empower labs to increase their throughput, improve sensitivity, and expand their testing portfolio to cover a wider range of food safety applications than ever before imagined.

Examples of the use of LC–MS/MS in ingredient profiling as well as the use of accurate mass incorporating the latest software tools for contaminant screening as well as the use of micro LC in food testing will be presented.

VENDOR SEMINAR: NOVEMBER 6, 2013 (18:30–19:30)

ELIMINATING A BOTTLENECK: HOW ADVANCED EXTRACTION TECHNOLOGIES IMPROVE THE SAMPLE PREPARATION PROCESS

Learn how to streamline your sample preparation with maximum speed and throughput for your everyday extraction tasks. Explore and discuss cutting-edge developments with distinguished scientists.

Optimization of pressurized liquid extraction (PLE) for rapid and efficient mineral oil extraction from cardboard packaging and dry foods

***Sabrina Moret**, Department of Food Science, University of Udine, Italy*

Determination of phenolic compounds in above- and underground organs of dropwort (Filipendula vulgaris Moench)

***Jarosław Leon Przybył**, Factuly of Horticulture, Warsaw University of Life Sciences, Poland*

VENDOR SEMINAR: NOVEMBER 6, 2013 (18:30–19:30)

THE TOXIMET SYSTEM – A REVOLUTIONARY TECHNOLOGY FOR THE ACCURATE, SIMPLE AND AFFORDABLE ANALYSIS OF MYCOTOXINS, AND OTHER FOOD & FEED TOXINS

Introduction to the ToxiMet System

***Raymond Coker**, ToxiMet Limited, Kent Science Park, Sittingbourne, UK; E-mail: ray.coker@toximet.com*

The *ToxiMet System* is a revolutionary technology for the measurement of mycotoxins in food. It is composed of three main elements: the *ToxiSep cartridge* that cleans up the sample extracted from the raw commodity; the *ToxiTrace cartridge*, on which the cleaned-up sample is immobilised; and, the *ToxiQuant instrument* which rapidly, accurately and *simultaneously* measures the concentrations of individual toxins which have been immobilised on the ToxiTrace cartridge. The ToxiQuant has been specifically designed to be operated by non-scientists, at ambient temperatures as high as 50°C. The results are reported on an interactive touch screen in less than 4 minutes.

The ToxiQuant instrument is composed of highly sophisticated, patent protected spectroscopic analysis hardware and software, together with chemometric algorithms which *simultaneously* identify and quantify the individual toxins immobilised on the ToxiTrace cartridge*, without physically separating the toxins*. This affordable, unique combination delivers outstanding accuracy, at sub-parts per billion levels.

Current applications of the System include the simultaneous quantification of aflatoxins (B1, B2, G1, G2) in edible nuts, rice, corn and figs, and ochratoxin A in dried vine fruit. Further applications will follow very shortly, including the analysis of Fusarium toxins (including zearalenone, deoxynivalenol and fumonisins) in wheat and corn.

The ToxiQuant produces quantitative data that is in excellent agreement with high performance liquid chromatography (HPLC) with an approximately 70% saving in cost.

ToxiMet has carried out extensive research into the ToxiQuant instrument's Limit of Detection (LOD) and Limit of Quantitation (LOQ) levels, using a statistical weighted linear regression method. The LOD and LOQ limits for individual aflatoxins in commodities analysed are well below the EU regulatory limits for these commodities (e.g. $AFLB_1$ LOD = 0.20 ppb, LOQ = 0.67 ppb).

The ToxiMet System is currently in the process of gaining AOAC certification.

Demonstration of the ToxiQuant Instrument

***Monika Szabo-Vezse**, ToxiMet Limited, Kent Science Park, Sittingbourne, UK; E-mail: monika.vezse@toximet.com*

The employment of the ToxiTrace cartridge, containing immobilised toxins, in combination with the ToxiQuant Instrument, to simultaneously measure each of the four individual aflatoxins, in four minutes, will be demonstrated. The simplicity of the ToxiQuant will also be illustrated using its especially designed interactive touch-screen.

VENDOR SEMINAR: NOVEMBER 6, 2013 (18:30–19:30)

AUTOMATION OF MYCOTOXIN ANALYSIS – AN AUTOMATED SYSTEM FOR MYCOTOXINS USING ON LINE IMMUNOAFFINITY CARTRIDGES IN CONJUNCTION WITH HPLC

Automation of mycotoxin analysis – An automated system for mycotoxins using On Line Immunoaffinity Cartridges in conjunction with HPLC

***Ria Rhemrev, Elizabeth Manning, Tilmann Bur**, R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany*

Automation and increasing efficiency play an important role in routine testing laboratories. R-Biopharm Rhone NL and R-Biopharm Rhone Ltd, have developed a patented, ON-LINE affinity cartridge for aflatoxins in collaboration with Spark Holland and Eurofins, Hamburg, which can be used prior to HPLC or LC–MS/MS. The cartridges are used together with the Symbiosis ^{TM} handling system from Spark, Holland and combine automated on line sample application with quantitative analysis of aflatoxin B_1. B_2 G_1 and G_2. The affinity cartridge contains a monoclonal antibody that is specific for aflatoxins B1, B2, G1 and G2, coupled to a hydrophilic polymer that can withstand high pressure. The technology is highly innovative and enables the cartridge to be incorporated directly on-line with an LC system. The affinity cartridge offers highly specific, sensitive, rapid and automated analysis for aflatoxins in a wide range of food matrices. Using the aflatoxin affinity cartridge, the sample application, washing and elution is performed on line for up to a maximum of 12 samples before the cartridge is automatically removed and replaced with a new cartridge. This level of reuse has been found to offer optimum cartridge performance and reduce the chance of interference or carryover.

The method has been validated with various products like cereal, peanut and dried fruit samples.

A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the affinity cartridge, reducing the time taken for subsequent sample clean up to almost zero. The use of an on line affinity cartridge reduces labour, consumables and solvents, whilst improving traceability, accuracy and reducing human error.

VENDOR SEMINAR: NOVEMBER 6, 2013 (18:30–19:30)

INSIDE TIPS ON IDENTIFYING, APPLYING AND INTERVIEWING FOR JOBS

VRS are the leading Scientific Recruitment Agency specialising in jobs within Analytical Chemistry, predominantly Mass Spectrometry & Chromatography (HPLC, GC, LC–MS, GC–MS, ICP–MS, IR–MS).
With office locations in the UK, Germany and US we provide unparalleled expertise to jobseekers and employers globally.

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This Career Advice Seminar is designed by VRS Experts to provide you with in-depth knowledge of careers available within the Analytical Chemistry (specifically Mass Spectrometry & Chromatography) and Life Science sectors across Europe. We will discuss how best to identify and apply for opportunities, providing guidance on writing CV's and covering letters. VRS will provide you with a valuable insight into the interview process with tips to maximize your success in securing your dream job! We will also invite your questions and encourage a friendly and informal discussion to help everyone to succeed in their career ambitions within this exciting industry.

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VENDOR SEMINAR: NOVEMBER 7, 2013 (13:15–14:15)

IS THERE HORSE IN MY MEAT BALL?
THE FUTURE OF LC–MS/MS IN MEAT SPECIATION AND ALLERGEN DETECTION

Is there horse in my meat ball?
The future of LC–MS/MS in meat speciation and allergen detection

Stephen Lock

Following the UK Food Standards Agency (FSA)'s announcement in January that horse and pig DNA had been identified in beef products sold by several supermarket chains, further testing across Europe and beyond has revealed widespread incidences of such food contamination. However, like allergen analysis as well most testing methods are based on detection of species-specific DNA in meat, using the polymerase chain reaction (PCR) – which does not detect or identify proteins. This is a concern because DNA can be easily disrupted or removed during standard meat processing and food manufacturing. As a result, horse tissue or other contaminants remain undetected in food samples, despite strong presence of the contaminating proteins. An alternative protein-based method, ELISA (enzyme-linked immunosorbent assay), can be used to complement DNA testing, but this method has limitations, including that it detects only one part of the protein and not multiple protein markers.

The LC–MS/MS-based methods presented offers a more accurate and reliable approach to meat speciation than PCR or ELISA-based techniques or other indirect methods, and also allows for the detection of veterinary drug residues in the same analysis, which is not possible by ELISA or PCR. The method is developed using an Eksigent micro LC system coupled with an AB SCIEX QTRAP® 5500 LC–MS/MS system and uses multiple reaction monitoring (MRM) to detect peptide markers for horse and is capable of providing sequence information by acquiring a product ion scan for each triggered MRM which can be used to further confirm the peptide's / proteins and therefore the species identity. Using the same extraction and LC–MS/MS method it is also capable of simultaneously of detecting veterinary drug residues by adding additional MRM experiments. The method has been shown to be capable of simultaneously detected phenylbutazone below 10 µg/kg as well as a 1% contamination of horse meat in beef. This approach offers food analysts the ability to detect multiple species and veterinary drug residues in a single approach which is not possible by any other technique to date

Further to this the same work flow used in species detection can also be applied to allergen detection and examples of the use of LC–MS/MS in allergen analysis in a variety of different matrices will also be shown.

VENDOR SEMINAR: NOVEMBER 7, 2013 (13:15–14:15)

BRUKER CORPORATION: LEADING SOLUTIONS FOR MONITORING FOOD SAFETY, QUALITY AND AUTHENTICITY

An Introduction to Bruker's Market Leading Chromatography and Mass Spectrometry Portfolio of Food Quality and Safety Testing Solutions

***Joe Anacleto**, Bruker Daltonics*

The globalization of the food supply chain poses increasingly difficult challenges to producers and governments tasked with maintaining the safety and quality of our food. With a constantly growing number of potential contaminants and a strong public demand for food quality, new stringent regulations are being introduced globally that escalate the need for advanced testing capabilities.

Modern systems based on gas and liquid chromatography coupled to tandem mass spectrometry are very well suited to meet the challenges of rapid screening, identification or quantification of trace level chemical residues in complex food matrices. In addition, inductively coupled plasma mass spectrometry is also an ideal tool for rapid, low level detection of inorganic continuants within food products. This presentation will provide an overview of Bruker's lab-based chromatographic and mass spectrometric systems and how they provide market leading performance, ruggedness and ease-of-use when used with our innovative software solutions.

FT–NIR Analysis of Food Products from Lab to the Process

***Alicja Spychala**, Bruker Optics, Poland*

Near Infrared Spectroscopy has been a well-established technique in the agricultural sector for decades and is recently becoming more and more important in the food industry. Modern multipurpose spectrometers can analyse both, liquid and solid samples and are the ideal tool for the non-destructive and rapid analysis of incoming raw materials and finished products throughout the entire manufacturing process.

The FT–NIR technology offers a lot of advantages over classical wet-chemical and chromatographic analyses. It is quick, cost-effective and safe, since no hazardous chemicals are used. It simply measures the absorption of near-infrared light of the sample at different wavelengths. The recorded NIR spectrum is characterized by overtones and combinations of the fundamental molecular vibrations of molecules containing C-H, N-H or O-H groups, making NIR spectroscopy first choice for the analysis of organic materials like edible oils, dairy products, condiments or meat products.

FT-NIR also avoids the typical error sources of the classical lab methods, e.g. during the sample preparation stage. With only one measurement, multiple components can be analysed in less than one minute. Although NIR spectroscopy is not a technology for trace analysis like for toxins, it will help the producer to constantly monitor the quality of the goods along the production chain - from checking the incoming raw materials, monitoring of intermediate products up to quality testing the finished product.

Beside lab and at-line analysis close to production FT–NIR is capable to analyse all kind of sample right in the process means on-line or in-line. The instrument is placed somewhere close to production and fiber probes or measurement heads are attached to it by fiber optic cables. The NIR light is send to the sampling point and back over up to 100m and multiple points can be controlled by one instrument. This allows an almost real-time monitoring of the production process. Rather than relying on single lab samples, plant

operators with access to frequent results are able to dramatically reduce in-process variation and adjust the process in time to avoid the production of out of spec product.

Food analysis by means of Total Reflection X-Ray Fluorescence (TXRF) spectroscopy – Application for quality, authenticity and food fraud control

Armin Gross* & *Hagen Stosnach, *Bruker Nano, Germany*

In this study the feasibility and restrictions of TXRF spectroscopy for the analysis of trace elements in food samples are evaluated. Main target of the investigation was to perform measurements with a minimum of sample preparation and operation costs.

The analysis of solid certified reference materials (NIST 8436 – Wheat flour, DORM-3 – Fish muscle, NIST 1515 – Apple leaves) has proven that an accurate analysis of the elements P, S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr and Pb is possible after a simple slurry preparation and quantification by means of internal standardisation.

Liquid nutritional products were analysed by means of TXRF, inductively coupled plasma atomic emission (ICP–AES) and mass spectrometry (ICP–MS). The results show good concordances between TXRF and ICP–AES/ICP–MS methods for the investigated elements P, Ca, K, Mn, Fe, Cu, Zn and Se.

In addition to quality control measurements TXRF offers a fast and easy solution for authenticity analysis as it was demonstrated by the analysis of a batch of Californian wine samples. Combined with additional analytical methods like NIR or MALDI–TOF the TXRF method can also be a strong tool for food fraud control.

Fast and Reliable methods for food authenticity, quality and safety control

Léa Heintz, *Bruker BioSpin, Rheinstetten, Germany*

The transfer of metabolomics approaches to food authenticity and quality control applications has rapidly expanded in recent years.

Thanks to its unmatched reproducibility and transferability properties, the Nuclear Magnetic Resonance (NMR) established itself as a unique and powerful tool for metabolomics analysis.

The NMR allows the simultaneous detection of very small changes in concentration of many compounds in mixtures. These changes can be observed both in untargeted and targeted mode while running a single experiment.

Standard operation procedures as well as extensive databases of authentic samples have been developed for fruit juice and wine analysis.

The features of these fast and fully automated methods will be described in detail.

With the WineProfiling method, over 50 relevant parameters are quantified simultaneously, amongst which alcohols, sugars, organic acids, amino acids, polyphenols and degradation parameters. Each individual compound concentration is compared to the reference concentration distribution of authentic wines in order to support interpretation of the results.

Furthermore, the method allows the prediction of the wine grape variety. Discrimination of geographical origin and vintage are also available for German Riesling and will be extended soon to other wine grapes.

It will also be demonstrated how in the untargeted mode, any deviation from the normal model can be detected, even for compounds that had not previously been identified.

Finally it will be shown how the procedures developed for juice and wine can be taken as models for many other food materials.

VENDOR SEMINAR: November 7, 2013 (13:15–14:15)

NEW APPROACHES FOR SAMPLE PREPARATION AND ANALYSIS BY MASS SPECTROMETRY

Agilent Technologies

Determination of PCDD/F and PCB with the new automated sample preparation system DECS

***T. Bernsmann*[1], *U. Möhlenkamp*[1], *P. Fürst*[1], *U. Aulwurm*[2], *M. Baumann*[2]**

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The major PCDD/F and PCB contamination cases which have occurred in the food and feed chain during the recent years demonstrate the need for fast and high throughput methods to identify and confirm non-compliant samples and to trace back the contamination sources. For this purpose, a highly efficient clean-up procedure is required to purify raw extracts prior to the final analytical separation and quantification.

With LCTech we developed a new method which focuses on the automation of our well-proven sample clean-up procedure using the automated DECS System (LCTech). The principle of the method is based on the clean-up of the acid stable PCDD/F and PCB on silica gel coated with sulfuric acid. A separation of the PCDD/F from PCB is subsequently performed on a Florisil column. For further purification, both eluates of the Florisil column are cleaned up on two different carbon columns which contain a different active carbon. The PCB fraction can be split into a group of non-ortho PCB, and a fraction containing the mono- and di-ortho PCB. This is important because the non-ortho PCB fraction includes PCB 126 and 169 which were assigned the highest toxicity factors of the PCB (WHO 2005). If PCB 126 is measured along with the other PCB, it may cause interferences, which can lead to a substantial overestimation of the PCB 126 concentration depending on the separation column. It is therefore essential to separate the non-ortho PCB from the other PCB. The PCDD/F fraction also needs to be cleaned up on a carbon column to separate matrix substances which may potentially interfere especially with the tetra-, penta- or hexa-CDD/F traces in the mass spectrometric analysis. The advantage of the automated clean-up procedure is that the columns are easy to handle and can be bought filled and ready-to-use. The whole process of column conditioning and clean-up of the sample extracts is done automatically in 97 minutes. The recoveries for each congener determined in a proficiency test material with the automated sample preparation system are in good agreement with the legislation requirements laid down in Commission Regulation (EU) No 252/2012 and range between the requested limits of 60% to 120%. A comparison study was performed on quality-control samples and food samples of animal origin to evaluate the robustness of the new automated sample clean-up system and also to compare the quantification using GC–HRMS and GC–MS/MS. All results demonstrate the suitability of the automated LCTech sample preparation system and the GC-MS/MS system for a fast and reliable routine analysis of PCDD/F and PCB congeners in foodstuffs and feedstuffs that meet the requirements of European Union legislation.

Fast and Fully Automated Multi-Residue Pesticide Screening in Fruit / Vegetable Extracts using a GC–Q/TOF

***Chris Sandy*[1], *Jennifer Gushue*[2], *Maithilee Samant*[2], *Hong Chen*[2], *Prerana Kapase*[2], *Joerg Riener*[3]**

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[2] *Agilent Technologies Inc., Santa Clara USA*
[3] *Agilent Technologies GmbH & Co., Waldbronn, Germany*

There is increasing demand to expand the scope of analytical methods employing GC/MS for food contaminants to include many hundreds of analytes. Although many laboratories have migrated their multi-residue pesticide methods from GC–MS to GC–MS/MS, as analysis suites become ever larger, concerns inevitably arise over instrument duty cycle. Another approach is to use a GC–Q/TOF system in full spectrum acquisition mode and to use the increased selectivity provided by high-resolution accurate mass

measurements in order to measure, theoretically, an unlimited number of compounds. The additional sensitivity and selectivity provided by the GC–Q/TOF is particularly relevant for the analysis of pesticide residues in foodstuffs prepared by the QuEChERS sample preparation technique, where large amounts of sample matrix may be present in the final extract.

This presentation will introduce the All Ions workflow for the screening of pesticide residues in various foodstuffs using a GC–Q/TOF and electron impact ionization in combination with a retention time locked GC method, backflush for increased method robustness and a new accurate mass spectral database of pesticides.

The All Ions workflow for accurate mass GC–Q/TOF is seamlessly integrated in to the Agilent Mass Hunter Qualitative software program and is similar to that of the All Ions workflow used for screening contaminants in foodstuffs with Agilent's Q–TOF LC/MS instrument. This new, comprehensive workflow for GC/MS accurate mass data uses Agilent's proprietary Personal Compound Database and Libraries (PCDLs) to identify compounds using their precursor (reference) and fragment ions. The workflow includes the use of a new accurate mass PCDL that contains more than 700 pesticides and begins by looking for molecular evidence by isotope pattern matching, followed by examination of the co-elution profiles of fragment ions of target compounds. The introduction of a novel Co-elution Plot and Co-elution Score to visualize and express the covariance of fragment ions with the precursor (reference) ion enables identification via accurate mass fragment ions. Unlike competitive approaches, the Co-elution Score not only uses just the retention time, but also the entire chromatographic peak information (including peak width and symmetry) to determine covariance of fragment ions and the precursor (reference) ion.

The combination of retention time locking and accurate mass measurements by the All Ions workflow provides more confidence in target compound identification and facilitates the reduction / elimination of potential false positives.

VENDOR SEMINAR: NOVEMBER 7, 2013 (14:30–15:30)

INNOVATIONS IN FOOD ANALYSIS: TIME-OF-FLIGHT MASS SPECTROMETRY IN COUPLING WITH SOPHISTICATED SEPARATION AND SAMPLE PREPARATION TECHNIQUES

Detailed food sample examination: One step target and non-target analysis of contaminants by GC–hr–TOF MS

***Jitka Zrostlíková*[1], Tomáš Kovalczuk[1], Kamila Kalachová[2], Lucie Drábová[2], Jana Hajšlová[2]**

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Today requirements in the analysis of food contaminants target at low ppb concentration levels and require confident confirmation of identity. At the same time, the methodics of sample preparation tend to be more time-efficient and universal, which results in less clean extracts and more potential matrix interference. Therefore new technologies of GC– and LC–MS analysis need to be implemented to meet the above requirements. New LECO multireflecting GC–TOF MS provides mass resolution 50,000 (at *m/z* 219) and mass accuracy 1 ppm at data acquisition rate up to 200 Hz. In this presentation the results from the analysis of pesticide residues and other contaminants in heavy food matrices will be demonstrated.

"Productivity and Simplicity" Automation of Sample Preparation workflows for the Food Laboratory

Oscar G. Cabrices

GERSTEL, Inc. USA, 701 Digital Drive, Suite J, Linthicum, MD 21090; Email: ogcabrices@gerstelus.com

Food Safety laboratories around the world are trying to find ways to minimize sample preparation and enhance productivity. The adaptation of modern GC/MS and LC/MS instrumentation is desired due to the high sensitivity and selectivity they provide. This presentation will describe how different sample preparation technique can be simplified and automated using the GERSTEL MPS autosampler for food residue analysis. Some of the applications that will be highlighted are:

- Derivatization and Extraction of Glyphosate and other polar pesticides from drinking water and food commodities
- Extraction and determination of mycotoxins in corn and feed samples
- **Determination of Total Fat, Saturated Fat, Monounsaturated Fat and Trans Fat Content in Food Samples**
- **Extraction and Detection Acrylamide in Brewed Coffee Samples**

Analysis of Herbal Teas for Pesticides and Biologically Active Compounds with QuEChERS and GC×GC–TOFMS

***Jack Cochran*, Michelle Misselwitz, Julie Kowalski**, *Restek Corporation, 110 Benner Circle, Bellefonte, PA, USA; E-mail: jack.cochran@restek.com*

The QuEChERS sample preparation method was applied to herbal teas, which are advertised to have important health benefits or even medicinal properties. The powerful separating power of GC×GC was combined with a TOFMS to screen for biologically active compounds in these tea extracts via full mass spectra. A GC×GC–TOFMS method was also developed to do pesticide analysis on the same QuEChERS extracts.

VENDOR SEMINAR: NOVEMBER 7, 2013 (14:30–15:30)

A NEW HIGH–SPEED GC–MS/MS SYSTEM FOR TARGETED AND UNTARGETED FOOD ANALYSIS

Fast, easy and efficient untargeted and targeted food analysis by using a novel high-speed GC–MS/MS system

***Peter Q. Tranchida*, Luigi Mondello**, *University of Messina, Italy*

The present seminar is focused on the use of a novel «unified» high-speed triple quadrupole mass spectrometer, under fast and very fast GC conditions. The MSMS device is capable of rapid operation under full-scan conditions, generating MS-database matchable spectra; additionally, the new instrument can produce MSMS spectra with frequencies of up to a 100 Hz. Moreover, the MS system can also be operated in the simultaneous full scan/MSMS mode. The latter characteristic makes untargeted and targeted analysis possible, in the same application. A series of fast GC–MSMS food applications will be shown, demonstrating the exceptional features of the instrument. The final part of the seminar will be devoted to the use of the tandem mass spectrometer under extreme GC conditions, namely those generated in comprehensive 2D GC experiments.

A novel GC–MS/MS system for highest sensitivity, aquisition speed and ease of use

Rebecca Kelting

For Food safety analysis sensitivity is one of the important factors. The GCMS–TQ8030 triple quadrupole mass spectrometer starting from the high efficiency ion source to the patented overdrive lens in front of the detector matches highest requirements. The advanced scanning speed protocol allows to acquire high speed data (20,000 amu/sec in scan mode, 600 transitions/s in MSMS). No cross talk is measured in MRM mode. Easy maintenance and user guiding pictures (MSNavigator in the GCMSsolution software) reduces downtime to a minimum.

VENDOR SEMINAR: NOVEMBER 7, 2013 (14:30–15:30)

INNOVATIVE ANALYTICAL TOOLS FOR MULTI-RESIDUE ANALYSIS: FROM ULTIMATE SENSITIVITY TO EFFICIENT DATA HANDLING AND INTERPRETATION

Waters

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Residue Analysis on the Crossroad: the Need of Non-Target Screening in Food and Animal Feed by UHPLC–HR–Q–TOF MS as Alternative for the Conventional Target Screening Approach

***J. Luetjohann**, F. Schreiber, J. Kuballa, E. Jantzen,* *GALAB Laboratories GmbH, Max-Planck-Straße 1, D-21502 Geesthacht; Email: luetjohann@galab.de*

Presently the prevailing method for determining pesticides, Vet drugs and other legislative relevant contaminants in food and animal feedstock is a targeted approach using LC/MS/MS or GC/MS/MS. The definitions of methodologies and its target compounds are mainly based on the juridical requirements as well as customer requests on known residuals of which relevance is mostly based on historical experiences.

New active substances, contaminants and substances, not in the mainstream of public awareness, are often overlooked hitherto the inherent methodology of targeted analytical technologies. In an increasingly globalised World with foodstuff and packaging materials from a multitude of suppliers and origins, a targeted screening will inherently risk of omitting potential unwanted and toxic contaminants.

The goal of our development project was to develop a sensitive non-targeted screening methodology for determination of pesticides, Vet Drugs and other contaminants in Food and Animal feedstock using high resolution UHPLC and QToF MS technology. The Screening Detection Limits (SDL) for these substances should be below the MRL and/or no more than 5% false negatives should be accepted.

After intensive investigations on routine screening equipment in the high resolution mass spectrometry range, the selection was made on a UHPLC QToF (Acquity UPLC and Xevo G2-S QToF, Waters Corporation). This UPLC–MS system uses the so-called MS^E technology with a large library which can rapidly identify and quantify targeted compounds using a sophisticated software approach. Furthermore and in the same sample run, unknowns can also be isolated and possibly identified using compound-specific characteristics, such as accurate mass, the identification of adducts, fragmentation patterns, retention time and isotope ratios.

The validation of this non-targeted screening method was carried out using the current SANCO document 12495/2011 and also the Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines (Community References Laboratories Residues) and was successful for more than 600 selected pesticides, 200 Vet Drugs and 250 other contaminants of relevance. The SDLs were in the range from 1 to 10 µg/kg. This novel approach in analytical work will in the near future replace the tandem MS systems, which will be continued in the usage for confirmatory analytical work.

New technology solutions to increase the scope of food analysis capabilities

***Sara Stead**, Waters Corporation*

Ensuring the quality and safety of the food supply in accordance with the ever increasing regulatory and consumer demands represents a significant analytical challenge. The focus of this presentation is to provide an overview of Waters recent technology developments designed to aid the work of the food analysis laboratory from the new Acquity QDa detector making MS accessibility a reality to ion mobility enabled QTof MS for non-targeted multi-residue screening in complex samples.

Informatics tools for streamlining the validation process

***David Wayland**, Waters Corporation*

Introduction to UNIFI and NuGenesis capabilities showing the application of processing and data reporting tools and the industry perspectives using data generated as part of an independent validation study (SANCO 12495) of the pesticide screening solution.

LECTURES

(L-1 – L-102)

L-1
NEW RESEARCH CHALLENGES IN FOOD QUALITY AND SAFETY CONTROL

Elke Anklam[1*]

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The main challenges for all stakeholders involved in the food safety and food security sector relate to the availability of high quality, nutritious, safe and functional food accommodating a range of lifestyles and incomes. As the access to safe food is a basic human right, it is important to supply safe and wholesome food in sufficient amounts to the society. Globalisation and international trade have drastically changed the way that food is produced, processed, transported, and consumed and raise the need for a more holistic and integrated approach to assure the safety of the food chain. With respect to food control, it is desirable to achieve a high quality and uniformity of test results throughout the EU and at global level, in particular in those areas where legal limits for hazardous substances in food need to be respected and enforced if necessary. The use of harmonized or standardized testing methods is mandated by EU legislation to guarantee that only safe food reaches the market. It is important to carry out underpinning research to understand the complexity of behaviour of food components through the whole food chain. This is of particular importance when there may be an impact of food on consumers' health such as food allergens or other harmful substances (natural or man-made) in food. It is likewise important to protect consumers against frauds. Food fraud is a criminal activity, which usually does not pose a threat to public health. However, this is not always the case as experienced even lately. It is a great challenge to predict potential problems related to food authenticity and frauds. Converging technologies (nano – biotech – ICT) are used to create food with new functionalities with the aim of increasing product safety, product shelf-life, health benefit, convenience of use, and profitability, and decreasing input of resources, and transaction costs. However, the overall impact of those novel technologies has not been thoroughly evaluated yet and is of concern to the general public. This presentation will elaborate on these challenges by using examples from the recent past and such to be expected in the near future.

Keywords: *Food Safety, Food Quality, Food Frauds, Harmonisation, Underpinning Research*

L-2
FOODOMICS: PRESENT AND FUTURE CHALLENGES IN FOOD ANALYSIS IN THE POSTGENOMIC ERA

Clara Ibañez[1], Alberto Valdes[2], Carolina Simo[3], Miguel Herrero[4], Elena Ibañez[5], Virginia Garcia-Cañas[6], Alejandro Cifuentes[7*]

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Nowadays, the use of advanced “omics” tools in food science and nutrition allows investigating topics that were considered unapproachable few years ago. This trend has generated a new discipline defined for the first time by our group as “Foodomics” [1–4]. Safety, quality and bioactivity of foods and food ingredients are investigated in Foodomics through the application and integration of advanced omics technologies, including genomics, transcriptomics, proteomics and/or metabolomics. The main goals of Foodomics are to improve consumers’ well-being, health and knowledge [1–4]. In this work, we will present the last results obtained in our laboratory following a Foodomics strategy to investigate the anti-proliferative effect of dietary polyphenols against human cancer cells. The present work will give additional information on how some dietary polyphenols are able to modulate specific metabolic pathways in the cancer cells, providing new evidences at molecular level on the antiproliferative effect of this type of compounds.

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Keywords: *Foodomics, metabolomics, polyphenols, cancer*

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L-3
RECENT AND FUTURE DEVELOPMENTS OF EU POLICY ON CONTAMINANTS IN FOOD

Frans Verstraete[1*]

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The EU legislation on contaminants in food fulfils two essential objectives: the protection of public health and removal of internal barriers to trade within the EU. Council Regulation (EEC) No 315/93 of 8 February 1993 laying down community procedures for contaminants in food is the framework for the Community action on contaminants. This Framework Regulation provides that food containing a contaminant in an amount which is unacceptable from the public health viewpoint shall not be placed on the market (food can only be placed on the market when it is safe). Furthermore it is foreseen that - contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain -in order to protect public health, maximum levels for specific contaminants shall be established where necessary; -the consultation of a scientific body (EFSA) for all provisions which may have an effect upon public health is mandatory. To reduce the presence of contamination in the food supply "prevention is better than cure". Therefore there it is important to encourage preventive actions such as good agricultural practice, good manufacturing practices, good storage conditions, use of improved sorting procedures etc… Prevention requires knowledge and acquiring knowledge requires research. Legislation on contaminants needs continuously be updated to ensure a continuous high level of human protection and to address the challenges with which the risk managers are faced. However there are changing trends in the EU policy on contaminants in food. The presentation will focus on the recent and expected future changes in EU-legislation on food contaminants. The following topics will be addressed while highlighting the challenges and the issues at stake: - mycotoxins: Alternaria toxins, citrinin, phomopsins, ergot alkaloids, T-2/HT-2 toxin, sterigmatocystin, masked (hidden/bound) mycotoxins, metabolites of mycotoxins. - plant toxins: pyrrolizidine alkaloids, tropane alkaloids, opium alkaloids, tetrahydrocannabinol, – process contaminants: acrylamide, MCPD esters and glycidyl esters, PAH, … – environmental contaminants, including POPs: Brominated flame retardants (BFR), perfluoroalkylated substances (PFAS), dioxins and dioxin-like PCBs, perchlorate, cadmium, inorganic arsenic, lead, (methyl) mercury – the impact of changing weather conditions on the prevalence of certain contaminants in the food chain and the consequences for EU-policy on contaminants – future issues.

Keywords: *Food contaminants, EU legislation*

L-4
CHALLENGES IN FOOD (AND HUMAN) LIPIDS´ ANALYSIS

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Lipids are a large and diverse group of naturally-occurring compounds that store energy, give structure to cell membranes, and, as ´molecular messengers´, they serve as signalling molecules. Considering foods nutritional value, lipids are one of the key components, they are e.g. a source of essential components such as polyunsaturated fatty acids (PUFA), they support absorption of fat soluble vitamins etc. For in-depth structural and quantitative characterization of various lipid classes and their distribution within food crops and/or products, instrumental platforms based on advanced chromatographic methods coupled with mass spectrometric detection represent currently the most challenging option.
In our studies, we focused on application of these novel strategies for monitoring of lipids changes during storage and processing, i. e. under conditions when they may undergo various degradative processes such as oxidation. Compounds generated through oxidation reactions are related to undesirable sensory and biological effects. In addition to cytotoxic and genotoxic compounds, free radicals, products of lipids peroxidation also co-oxidize some vitamins, and thereby impair the nutritional quality of the foods. A high number of methodologies enabling determination of both primary and secondary oxidation products has been developed and implemented. However, some of these classic approaches such as peroxide value illustrating early stages of lipids oxidation, are highly empirical and their accuracy is rather questionable since the results vary with details of the procedure. Also thiobarbituric acid (TBA) test often applied for measurement of the extent of lipids oxidation has been criticized as being non-specific and insensitive for the detection of low levels of malonaldehyde. Not surprising that introduction of modern instrumental techniques that enable rapid obtaining of more comprehensive and specific information is urgently needed.
In addition to introduction of modern analytical methods for assessment of lipids qualitative parameters, we also were concerned with introduction of novel non-target screening strategies enabling lipids authentication. This presentation involves several case studies documenting challenges in lipids analysis:

- Simple and fast sample preparation strategy based on partition in ternary solvent system enabling, in a single step, fractionation of fish lipid classes according their polarity.
- Application of an ambient high resolution mass spectrometry (HRMS) employing direct Analysis in Real Time (DART) ion source for an assessment of lipids quality with regards to content of primary and secondary oxidation products
- Authentication of food lipids using fingerprinting / profiling strategy based on DART-HRMS, lipidomics
- Employing supercritical fluid chromatography (SFC) coupled with high definition mass spectrometry (HDMS) for analysis of frying oils and lipids isolated from human adipose tissue.

L-5
COMBINED (E)SEM AND ICP-MS APPROACH FOR THE SUCCESSFUL QUALITATIVE AND QUANTITATIVE ANALYSES OF METAL MICRO- AND NANO-PARTICLES IN FOOD PRODUCTS

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The food sector is going to gain many potential advantage benefits from emerging nanotechnology applications, but there are still a lot of doubts and not deeply understood aspects concerned with health and safety issues for consumers. In this context, inorganic nanoparticles such as metals and metal oxides are considered to be the most hazardous being non-biodegradable, and potentially they may be retained for a long time leading to an accumulation. Hence, even though a range of methods for the detection and characterization of NPs are available [1], clearly it is necessary to improve the knowledge of the ways for correctly measuring them also with the final aim of a future regulation or risk assessment approach [2]. We have developed and validated new analytical methods based upon the use of a combined SEM and ICP-MS approach for the qualitative and quantitative analysis of metal micro-nanoparticles in food products. Attention was paid to the development of a sample preparation protocol aimed at avoiding artifact formation (i.e. particle aggregation or particle agglomeration). For this purpose, precooking/defatting steps (where necessary) were combined with enzymatic treatments and membrane filtration. In addition, the method was conceived in such a way to obtain a single filter suitable for both ICP-MS and SEM-EDS analyses, in order to match the information coming from the different analytical techniques related to the same sample. The ICP-MS method was validated in terms of linearity (0.8–80 µg/g and 0.09–9 µg/g for Fe and Ti respectively), quantification limits (0.73 µg/g for Fe and 0.09 µg/g for Ti), repeatability (RSD% equal to 10% for Fe and 20% in wheat matrix as an example) and extraction recoveries (93±2%–101±2%). To our knowledge, this is also the first example of a method based on SEM-EDS measurements validated according to European guidelines for particle detection and quantification in real food samples, working in a dimensional range from 1 to 100 µm with an estimated error in size determination equal to 0.5 µm. The potential of an ESEM–FEG instrument was then exploited for a better investigation of the morphology and low dimension distributions of particles in food ingredients and finished products having the nanoscale level as a target [3]. Finally, these analytical strategies were successfully applied for the quantitative determination and the morphological and dimensional characterization of particles isolated from different raw materials and food products, like common wheat, semolina, pasta and bakery products.

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Keywords: *Metal micro-nanoparticles, food, inductively coupled plasma-mass spectrometry, environmental scanning electron microscopy, sample treatment*
Acknowledgement: *Barbara Melegari (Barilla, Parma, Italy); Alessandro Mangia (Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Università degli Studi di Parma, Parma, Italy).*

L-6*
MYCOTOXIN MAPPING OF 960 GLOBAL BEER SAMPLES USING DIRECT MULTIPLEX MICROSPHERE IMMUNOASSAYS

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Beer is one of the oldest alcoholic beverages known in the world. By using instrumental analysis, remnants of beer-like products were found in ancient pottery jars dating more than 7,000 years back. With a total global beer consumption of 189 billion litres in 2011, beer is the most popular alcoholic beverage. Nowadays, craft beer is increasingly popular and is often referred to as “the new wine”. New micro-breweries are emerging rapidly and they claim a bigger market share every year. These new breweries are very creative and responsible for a whole range of new beer styles. In 2012 the database of a globally respected beer rating website (www.ratebeer.com) contained 184,000 unique beers which could be divided over 73 original beer styles. Regardless of the style, the four basic ingredients of beer are; water, barley, hops and yeast. Barley, and to a lesser extent hops, can be contaminated with mycotoxins. They are very stable and, to a certain extent, can survive the very stringent brewing process. During steeping, germination and kilning contaminating fungi are still able to grow and produce mycotoxins. This is also the process where seedlings most likely conjugate certain mycotoxins into masked forms. The Multi-Analyte Profiling technology used in our research features color-encoded microspheres. Using this suspension array format we have built a fast direct inhibition immunoassay for the simultaneous detection of the mycotoxins aflatoxin B1 (AFB1), fumonisin B1 (FB1), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEA) and T-2 toxin (T-2). The specific monoclonal antibodies were coupled to the microspheres. For the detection we created unique fluorescent reporter molecules by the conjugation of the aforementioned mycotoxins to R-Phycoerythrin. Both flow cytometry and planar array formats were used during the development of this assay. The developed assay was applied to 960 international beer samples covering 58 beer styles, originating from 36 counties of which 45% were craft-brews. Based on the mapping results, as well as style and origin, 170 beers were selected for confirmation by a beer dedicated multi-mycotoxin LC–MS/MS method. This multi-LC-MS/MS method detects the same 6 mycotoxins, as well as their available metabolites and masked forms. As expected, DON and DON3G were the main contaminants. In some beers DON (most times combined with DON3G) concentrations easily exceeded the tolerable daily intake. Often the level of DON3G exceeded the level of DON with an absolute maximum of 1109 ng/ml DON3G compared to 363 ng/ml DON in one beer. FB1 was especially found in Southern European adjunct beers and in South African home-brewed beers with a maximum of 50 ng/ml. In some of the South African home-brews the presence of AFB1 was confirmed by the multi-LC-MS/MS method. Further confirmations are still pending. This fast and robust multiplexing assay was also validated as a screening assay for barley.

Keywords: *Mycotoxins, beer, microspheres, flow cytometry, planar array*

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L-7*
WINE AUTHENTICATION: METABOLOMICS FINGERPRINTING AS A TOOL FOR DISCRIMINATION AND CLASSIFICATION OF RED AND WHITE WINES ACCORDING TO VARIETIES OF GRAPE

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Nowadays, interest in food authenticity has become more and more important. Therefore, development of analytical methods for the detection of adulteration and assessment of authenticity of food and beverages is vital; even more if this product is very much appreciated by the consumers. This is the case of wine. Wine is made of the fermented juice of any of various kinds of grape. Different varieties of grapes and strains of yeasts produce different types of wine, and the final product may contain tens of thousands of chemical compounds in amounts varying from a few percent to a few parts per billion. However, the adulteration has been commonly described due to its high cost. For example, wine fraud is a form of fraud in which wines are adulterated, usually with the addition of cheaper products, the substitution of labels and/or incorrect declaration of origin. With these premises, it is vital the applicability of "omics" science, in this case metabolomics [1, 2]. The metabolomics fingerprinting approach, aiming at global analysis of numerous targeted or non-targeted low molecular compounds (metabolites) in a biological sample, has recently applied to diverse research areas, such as medicine, foods, plants…etc. Furthermore, the applicability of metabolomics has been related to recent advances in analytical techniques, such as mass spectrometry coupled to liquid chromatography. In this research, in order to avoid any possible discrimination of metabolites a direct injection of 358 wine samples, provided by Bundesinstitut für Risikobewertung (BfR), was carried out. In parallel, generic settings were applied using a UHPLC–Triple TOF 5600 (AB Sciex) during the method development in order to obtain wine profiles containing as many compounds as possible. Consecutively, data analysis involved the use of various chemometric tools, such as Marker View (AB Sciex) and SIMCA (Umetrics). In this research, supervised pattern recognition techniques, PCA (Principal Component Analysis) was studied. The PCA-DA, discriminant analysis, of metabolomics fingerprints revealed clear differences between varieties of grape in red wine, as well as for the classification of white wine according to varieties of grape. As an example, the identification of markers showed that the most significant Tempranillo marker was 7,8-Dihydrovomifoliol 9-rhamnosyl-(1-6)-glucoside, precursor of aroma. These markers were identified using complementary tools of PeakView (AB Sciex), such as Formula Finder (Elemental composition), IDA explorer (MS/MS pathway) and different libraries available on-line.

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Keywords: *Wine, Metabolomics, LC–HRMS, Authenticity*

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L-8
BIOASSAYS FOR BIOMARKERS: TOOLS FOR DETERMINING HUMAN EXPOSURE TO FOOD TOXINS

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We are exposed to multiple chemical threats through food, water and the environment. Many excellent techniques have been developed to measure these contaminants in feeds, foods and water and indeed human exposure has also been measured in some cases. The great difficulty with detecting exposure in humans in that traditional analysis to detect compounds and their metabolites will. only give an indication of very recent exposure. The importance of having tools for detecting indirect measurements of longer term exposure has now been realised and the use of biomarkers in exposure studies is now a rapidly growing field in food safety. Biomarkers can be considered to be chemicals, metabolites, susceptibility characteristics, or changes in the body that relate to the exposure of an organism to a chemical compound. Biomarkers have the ability to identify if an exposure has occurred the route of exposure, the pathway of exposure and the resulting effects of the exposure. An overview of the means of identification of biomarkers to a range of chemical contaminants will be presented and how they can be implemented to understand better the consequences of low level exposure to mixtures of substances.

Keywords: *Biomarker, chemical, exposure, food safety*

L-9
RECENT TRENDS IN APPLICATION OF MOLECULAR BIOLOGY BASED METHODS IN FOOD ANALYSIS

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For more than 15 years modern DNA-based techniques are used for official food control purposes such as the GMO detection. The invention of the polymerase chain reaction (PCR) a decade ago provided analysts with totally new tools for the specific amplification of the inherited material. Based on the high specificity of the PCR process, individual species/races/breeds can be distinguished. The PCR process is also very well suitable for automation and standardisation. Consequently, several PCR-based methods for various purposes such as species identification, GMO and pathogen detection are globally applied in food control and recognized as international standards (ISO). Recently, new DNA sequencing approaches have been developed, called 'Next-Generation Sequencing' (NGS). Due to the high demand for technologies that parallelize the sequencing process, producing thousands or millions of sequences concurrently, companies have developed respective instruments for ultra-high-throughput sequencing. NGS can be used both for metagenomics studies and the detection of sequence variations within individual genomes, e.g., single-nucleotide polymorphisms (SNPs), or structural variants (such as the 16S RNA). First applications have been also reported to identify microorganism and/or species-specific DNA in food samples. Beside those sophisticated applications, DNA molecules are also used as aptamers, single-stranded DNA or RNA molecules, which are able to form a tri-dimensional structure similar to antibodies, enabling those molecules to act according the same principles as antibodies, binding to specific domains on the surface of the antigen of interest. The presentation will give an overview about the current status and the capabilities of DNA-based methodologies suitable for food/feed control and will discuss it in the context of legal requirements and harmonisation among laboratories applying such technologies.

Keywords: *Food traceability, food authenticity, food analysis, PCR, DNA*

L-10
DETECTION OF PALYTOXIN-LIKE COMPOUNDS BY AN INHIBITION IMMUNOASSAY USING A MICROSPHERE-FLOW CYTOMETRY SYSTEM SUPPORTED BY IT–TOF ANALYSIS

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Palytoxin (PLTX) is a complex marine toxin which has been described as one of the most potent non-protein compounds known. PLTX was firstly isolated from the zoanthid genus Palythoa and later from the dinoflagellate genus Ostreopsis. Many PLTX analogs have been described to date. The presence of PLTX or PLTX-like compounds in seafood poses a serious threat to human health due to its toxicity and potential lethality, underlining the need to develop useful and rapid detection methods. Mouse bioassay and Liquid Chromatography Mass Spectrometry (LC–MS) are the most employed methods for have been employed for PLTX detection, but no validated and widely accepted protocols for PLTX detection and quantification are available. We have developed an immunodetection method for PLTX and/or PLTX-like compounds based on the use of internally encoded fluorescent microspheres coupled to flow cytometry detection (Luminex 200™). Firstly, PLTX was covalently bound to the surf ace of the microspheres. The immunoassay consisted of the competition between the PLTX attached to the microsphere surface and free PLTX present in the calibration curve or the sample for binding to a PLTX specific monoclonal antibody. The amount of primary antibody bound to the microspheres was quantified by a secondary antibody labeled with phycoerythrin. This method displayed a dynamic range of 0.6–7.5 nM with a maximal half inhibitory concentration of 1.5 nM. Samples from Palythoa tuberculosa and Ostreopsis siamensis were analyzed by this immunoassay after a simple extraction procedure yielding positive results for PLTX-like compounds, which was upheld by a neuroblastoma cytotoxicity assay. The profile of PLTX-like compounds present in some of these samples was studied by LC-MS using an Ion Trap-Time of Flight analyzer with an ElectroSpray Ionization (ESI) interface (LCMS–IT–TOF) in order to explore antibody cross-reactivity with PLTX-like toxins. LCMS–IT–TOF was employed to determine the number of molecules present in these samples based on UPLC separation/ mass spectrum. The results confirmed that this immunoassay is capable of detecting PLTX and other molecules of the PLTX group. This microsphere-based immunoassay provides a fast, easy-to-use screening method able to detect PLTX-like compounds in samples processed by a rapid extraction procedure.

Keywords: *Palytoxin, seafood, flow-fluorimetry detection, Luminex bead-based array, LC-MS-IT-TOF.*

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L-11
USE OF MONOCLONAL ANTIBODIES IN THE DEVELOPMENT OF SENSITIVE DETECTION ASSAYS AND THERAPEUTICS FOR BACTERIAL TOXINS

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Botulinum neurotoxins (BoNTs) and shiga toxins from Escherichia coli are some of the most potent toxins to humans. While BoNTs could be likely targets for use in intentional adulteration of food or animal feeds and are thus classified as Select Agents, shiga toxin-producing E. coli (STEC) could cause severe disease and has been the subject of massive food recalls. Currently, there is an intense research effort to develop sensitive detection tools, vaccines and therapeutics for these bacterial toxins. In our laboratories, high-affinity monoclonal antibodies (mAbs) have been developed for the sensitive detection of BoNTs and shiga toxins in traditional ELISA and new electrochemiluminescence type immunoassays. Detection limits of these new immunoassays fall within the pg/ml range, well below those of standard assays for BoNTs and shiga toxins from STEC. We have also determined the detection sensitivity of these assays in complex matrices of food and sera samples. Equally important, our mAbs have been tested for their ability to confer passive protection against botulism and shiga toxin in rodent intoxication models. A better understanding of the biology of toxins in animals and the factors that affect their toxicity, coupled with the development of more sensitive detection and simpler diagnostic tests, would be invaluable for advancing food safety and protection.

Keywords: *Botulinum neurotoxins, shiga toxins, monoclonal antibodies*

L-12
LESSONS LEARNT FROM THE CONffIDENCE PROJEKT: CONTAMINANTS IN FOOD AND FEED – INEXPENSIVE DETECTION FOR CONTROL OF EXPOSURE

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The presence of potentially hazardous chemicals in food remains a major concern among consumers. Recent food contamination incidents, e.g. regarding aflatoxin M1 in milk in Serbia, Romania, Croatia and The Netherlands through feeding of contaminated maize from Balkan countries certainly contribute to fears about the safety of food.
Currently, a variety of analytical test methods is used to help ensure the safety of food and feed in Europe, both for goods produced in the EU and imported from third countries. Many of these methods are tedious and time consuming and require sophisticated and expensive instrumentation.
The CONffIDENCE project aimed to further improve food and feed safety in Europe and beyond by the development of faster and cost-efficient screening and confirmatory methods for the detection of a wide range of chemical contaminants in different food and feed commodities. These methods will not only save precious time in ever faster production cycles, but will also permit more food/ feed samples to be monitored due to the lower costs per test. In combination with the broadened spectrum of detectable residues and contaminants the CONffIDENCE project has significantly increased food safety in Europe.
Within CONffIDENCE, rapid and simplified multi-methods have been developed for:

- persistent organic pollutants: PCB's, brominated flame retardants, PAH's
- perfluorinated compounds: PFOS, PFOA, FOSA
- pesticides: dithiocarbamates, paraquat
- antibiotics: tetracyclines, sulphonamides, quinolones, chloramphenicol, tylosin
- coccidiostats: lasalocid, monensin, narasin, salinomycin, nicarbazin and diclazuril
- heavy metal speciation: inorganic arsenic, methylmercury
- alkaloids: ergot, pyrrolizidine and tropane
- marine biotoxins: PSP, DSP, ASP, palytoxin and tetrodotoxin
- mycotoxins: DON, zearalenone, fumonisins and T-2/HT-2

in products such as seafood, fish feed, cereal-based food and feed, dairy products, vegetables, honey and meat. A balanced mix of novel multiplex technologies has been utilized, including dipsticks, flow cytometry with functionalized beads, optical and electrochemical biosensors, metabolomics-like comprehensive profiling, ambient MS and NIR hyperspectral imaging. The methods have been validated in-house and through small- or full-scale collaborative studies. Specific validation schemes for screening tests have been designed and successfully applied to a number of different methods. Moreover, international surveys have been organized that contribute to exposure assessment.
The consortium consisted of 16 partners from 10 European countries, representing 8 research institutes, 5 universities, 2 large food and feed industries and 1 SME. CONffIDENCE started in May 2008 and was finished in December 2012. It was coordinated by RIKILT – Institute of Food Safety, The Netherlands.
In the presentation, key results from CONffIDENCE will be presented. Among others key aspects regarding the validation experiments for screening tests will be discussed.
Website: www.conffidence.eu

Keywords: *Food, feed, chemical contaminants, rapid methods, screening methods*

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L-13
A RAPID MULTIPLEX ELISA FOR PYRROLIZIDINE ALKALOID DETECTION IN HONEY AND FEED

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Pyrrolizidine alkaloids (PAs) are a group of naturally occurring small molecular weight compounds produced as secondary metabolites by plants. They are mainly present in plants in the Boraginaceae, Asteraceae, Orchidaceae and Fabaceae families. More than 660 PAs and PA n-oxides have been identified in over 6,000 plants, and about half of them exhibit hepatotoxicity. PAs are known to cause livestock losses and can enter the human food chain as contaminants in grain, honey, milk, offal and eggs. To date there are no international regulations of PAs in feed or food and no equivalence of toxicity within this large class of compounds has been achieved. Though due to their profound biological effects the European Food Safety Authority (EFSA) are reviewing PAs as emerging toxins on behalf of their potential as feed and food contaminants. The current state-of-the-art in the detection of pyrrolizidine alkaloids is based on multiple toxin detection using gas chromatog raphy (GC) or liquid chromatography coupled with mass spectrometry (LC/MS). Therefore for any bioanalytical approach to be comparable to the state of the art it must be able to demonstrate a relatively cost-effective, rapid, multi-analyte screening model for this analysis. Protein conjugates, employing various conjugation strategies, were synthesised as immunogens for antibody production for the detection of senecionine, lycopsamine, heliotrine and monocrotaline type PAs in order to detect as many structurally similar alkaloid compounds. Polyclonal antibodies were successfully produced for the first three PA groups and these were characterised for sensitivity and specificity by enzyme immunoassay. The sensitivity of the antibodies produced for each group was pg to ng / ml levels. A multiplex rapid ELISA with a simple extraction procedure was developed and validated for the detection of these PAs and their N-oxides in honey and feed. The detection capability of the qualitative assay was $\leq$ 25 µg/kg and a comparison by multi-analyte LC-MS method displayed an effective correlation with analysed real samples down to a level of 10 µg/kg. A bioanalytical survey utilising the method for the detection of PAs in 146 honey samples from 17 countries revealed that 5 countries throughout Europe demonstrated levels of PA greater than 10 µg/kg in honey which indicates that PA contamination is not isolated to a specific region.

Keywords: *Plant alkaloids, pyrrolizidine, antibody, ELISA*

L-14
FAST SCREENING OF RESIDUES, CONTAMINANTS AND ADULTERANTS BY FLOW INJECTION–MS(/MS)

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Liquid chromatography with mass spectrometry is the most widely used technique for the determination of residues and contaminants in the food chain. It is sensitive, selective, and provides quantitative results. In addition, many analytes can be determined in one run which is very effective and efficient. However, there are situations where generic LC–MS based analysis is troublesome or inefficient. Examples include highly polar analytes (not retained in reversed phase LC) and samples in which the analytes co-occur at low and high levels (requiring additional injections of diluted extracts). In the fore mentioned cases direct MS detection can be advantageous in terms of cost and speed. Flow injection – MS(/MS) is a feasible option and turns out to be equally or even better suited compared to ambient MS in most cases. This will be illustrated and discussed through different types of applications:

1) Determination of highly polar analytes such as certain pesticides (e.g. chlormequat, glyphosate, fosethyl-Al, ethephon, maleic hydrazide). These pesticides require dedicated single residue methods. Including them in the routine scope would substantially increase the capacity demand on LC-MS instrumentation. Furthermore, special LC columns are required which often lack robustness and make chromatography the weakest link of the method. Flow injection–MS eliminates such problems, is much faster (30 sec) and, with the use of isotopically labeled internal standards, acceptable quantitative results can be obtained.

2) Dietary supplements. Here the use of flow injection combined with full scan high resolution MS is shown to be valuable as rapid screening method for the detection of compounds that may result in harmful effects to the consumer, such as natural toxins or synthetic adulterants (pharmaceuticals).

3) Food fraud: Rapid targeted detection of melamine by flow injection – MS/MS and non-targeted detection of protein adulterants by flow injection – full scan high resolution MS.

4) Agriforensics. Rapid detection of illegal pesticides through surface swaps taken at farm sites, or swaps from fruits labeled as organically grown. A comparison with ambient mass spectrometry as alternative option will be made.

Keywords: *Rapid methods, polar pesticides, plant toxins, fraud, agriforensics*

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L-15
NEW DEVELOPMENTS IN WINE ANALYSIS EMPLOYING ^{1}H-NMR SPECTROSCOPY

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The 1H-NMR analysis of aqueous matrices, such as wine, is especially challenging due to the dominant water signal as well as ethanol signals in the fingerprints masking interesting information. To expand the dynamic range for the analysis of minor elements, a new methodology is to suppress the water/ethanol signals using specific pulse sequences. NMR spectroscopy is currently employed to characterize wine in terms of a targeted and non-targeted analysis. Identification of different key wine ingredients and their quantification is possible by 1H-NMR spectroscopy in a few minutes. Furthermore the capability to detect known adulterants, but as well the ability to detect further abnormalities, and the assessment of challenging authentication parameters (grape variety, origin, vintage) make this technique of utmost interest for quality control, research and control institutions. Although very interesting results are obtained and have been presented so far, typically feasibility or research studies are undertaken within one laboratory on one instrument exclusively, which restricts validation possibilities, particularly concerning the non-targeted approaches. Validation of the whole analytical procedure including statistical data evaluation and consistency of the measurement over time, instruments and laboratories is, however, essential for routine application and in official control. Therefore the use of non-targeted fingerprinting approaches is due to actual missing validation strategies still restricted for official control purposes, thereby offering complex challenges for the scientific community. Powerful commercial solutions are currently available which unify measurements from different instruments of the same type and vendor by using a standardized operating procedure (including sample preparation and measurement protocol), which allows evaluation of the data with the same statistical models. However, in these cases the measurement procedures, the reference data (data base), its assessment and evaluation, are marketed as intellectual properties. The use or the acceptance of such proprietary methods or measurement procedures in standard setting organizations (Codex Alimentarius as well as International Organization for Wine and vine, OIV) is limited at this stage and are therefore currently not used in official consumer protection. Commercial developments will be presented and discussed as well as own research results on wine authentication by 1H-NMR spectroscopy.

Keywords: *NMR spectroscopy, wine, analysis, authenticity*

L-16
ANALYSIS OF NANOPARTICLES IN FOOD: FROM CHALLENGE TO ROUTINE?

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A number of recent reports and reviews have identified the current and short-term projected applications of nanoparticles for food and beverages. These include nano-sized or nanoencapsulated ingredients and additives for food, beverages, and health-food applications as well as the use of engineered nanoparticles for the improvement of food contact materials with view to mechanical properties, gas permeability or antimicrobial activity. Although potential beneficial effects of nanotechnologies are generally well described, their potential (eco)toxicological effects and impacts have so far received little attention. A prerequisite for toxicological, toxicokinetic, migration and exposure assessment is the development of analytical tools for the detection and characterisation of nanoparticles in complex matrices such as food. Given the huge diversity of engineered nanoparticles (and conventional materials with a size distribution extending into the nano-range) for use in the food and feed sector in terms of chemical composition, size, size distribution, surface modifications and potential interaction with food matrix components (e.g. proteins) this is a challenging task requiring tailored solutions.
In recent years a number of institutes and projects (i.a. NanoLyse) have started to develop methods that are capable of detecting and quantifying nanoparticles in food matrices. The developed approaches include sample preparation aspects, imaging techniques such as electron microscopy, separation methods (e.g. field flow fractionation, hydrodynamic chromatography, centrifugation) and detection/characterisation techniques (e.g. light scattering, mass spectrometry). In 2011, the European Commission published a recommendation for a definition of a nanomaterial which was adopted by regulators for the implementation in European legislation. First respective regulations come in force for the labelling of cosmetics (2013) and food (2014). This triggers the need for reliable and validated methods that deliver traceable results and allow the decision if a product contains nanomaterials according to the EC definition.
The presentation will review recently developed approaches and evaluate their fitness for purpose with view to analytical and legislative requirements as well as for routine use in the monitoring of actual food samples. Method validation is a relevant issue (involving the availability of suitable reference materials) and results of first interlaboratory studies will be assessed.

Keywords: *Nanoparticles, analytical techniques, detection, characterisation, NanoLyse.*

L-17
CHARACTERISATION OF TITANIUM DIOXIDE IN FOOD AND FOOD ADDITIVES

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The Netherlands Titanium dioxide is a common food additive and registered in the EU as E171. As a pigment, titanium dioxide is used to enhance the white colour of certain foods, such as dairy products and candy. It also lends brightness to toothpaste and some medications. Not much is known on the titanium dioxide content and the nano-sized fraction therein, in foods and consumer products. In this study the size distribution of a series of synthetic, food grade titanium dioxide additives (pure E171) is determined using scanning electron microscopy (SEM), asymmetric flow field-flow fractionation with inductively coupled plasma mass spectrometry (AF4-ICPMS), and single particle ICPMS (sp-ICPMS). In this study 7 food-grade TiO_2 materials, 24 food products and 3 toothpastes were investigated for their titanium dioxide content and the size distribution of these titanium dioxide particles. Analyses with SEM showed that the food-grade TiO_2 materials are very much alike and have similar size distributions with primary particles in the range of 60 to 300 nm. About 10% of the particles in these materials had sizes below 100 nm. Of the 27 food and personal care samples that were tested, 24 showed detectable amounts of titanium while 19 products showed amounts of titanium higher than 0.1 mg Ti/g product. The highest concentration was found in a chewing gum that contained 5.4 mg Ti/g product which translates to 9.0 mg TiO_2/g product. There was a good correlation between the TiO_2 content in the products as determined with AF4–ICPMS and the total-titanium content of the product. Number-based size distributions were determined directly from the sp-ICPMS analysis and indirectly (af ter transformation from mass- to umber-based) from the AF4-ICPMS analysis. It was found that the number-based-size distributions determined for TiO_2 particles in the food and personal care products are comparable with those found for the food-grade TiO_2 materials with the same method. The number of particles with sizes below 100 nm in the food and personal care products was about 10%, comparable with the results found for the TiO_2 materials with SEM as well as with AF4–ICPMS and sp–ICPMS. Since this size distribution is expressed on a particle number basis, it allows us to decide whether the studied food additives should be labelled as a nanomaterial according to the recommendation of the definition of a nanomaterial (2011/696/EU).

Keywords: *Nanoparticles, titanium dioxide, electron microscopy, field flow fractionation, single particle ICPMS.*

L-18
DETECTION AND CHARACTERIZATION OF SILVER NANOPARTICLES IN CHICKEN MEAT

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With the increasing use of nanotechnology in food and consumer products, there is a need for reliable detection and characterization methods for nanoparticles (NPs) in complex matrices. Asymmetric flow field-flow fractionation (AF4) coupled to inductively coupled plasma mass spectrometry (ICP–MS) is a highly promising method for this purpose. However, the number of publications regarding sample preparation of NPs in organic matrices including food stuffs, tissues and cells for AF4–ICP–MS is still limited. Silver nanoparticles (AgNPs) are presently one of the most frequently used nanomaterials in consumer products related to food, such as food storage containers and dietary supplements. In the presented work AgNPs were incorporated into chicken meat to illustrate a possible scenario where AgNPs may migrate from an antibacterial food contact material into meat. A method of analysis was developed based on enzymatic digestion followed by AF4 - ICP–MS fractionation and detection. The method was validated in terms of precision, reproducibility, linearity and limit of detection / quantification. In addition, single particle ICP–MS was applied for determination of the number-based particle size distribution of AgNPs in collected fractions. The talk will describe which methodological steps were necessary, and highlight the challenges that had to be addressed, in order to develop an appropriate sample preparation method for AgNPs in meat.

Keywords: *Silver nanoparticles, food, field flow fractionation, single particle ICP–MS, enzymatic digestion*

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L-19
DIGESTION, ORAL UPTAKE, AND TOXICITY OF SILVER NANOPARTICLES – IMPACT ON RISK ASSESSMENT

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Orally ingested nanoparticles may overcome the gastrointestinal barrier, reach the circulatory system, be distributed in the organism, and cause adverse health effects. However, ingested nanoparticles have to pass through different physicochemical environments, which may alter their properties before they reach the intestinal cells. Therefore we hypothesised that a new in vitro digestion system may simulate the changes during the digestion process. In our study, silver nanoparticles are characterized physicochemically during the course of artificial digestion to simulate the biochemical processes occurring during digestion. Their cytotoxicity on intestinal cells was investigated using the Caco-2 cell model. Using field-flow fractionation (A4F) combined with dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS), we found that particles only partially aggregate as a result of the digestive process. Cell viabilities were determined by means of CellTiter-Blue® assay, DAPI-staining and real-time impedance. These measurements reveal small differences between digested and undigested particles (1 to 100 µg/mL or 1 to 69 particles per cell). Our findings suggest that silver nanoparticles may indeed overcome the gastrointestinal juices in their particulate form without forming large quantities of aggregates. Consequently, we presume that the particles can reach the intestinal epithelial cells after ingestion with only a slight reduction in their cytotoxic potential. Our study indicates that it is important to determine the impact of body fluids on the nanoparticles of interest to provide a reliable interpretation of their nanospecific cytotoxicity testing in vivo and in vitro. The impact of the new findings on risk assessment of silber nanoparticles will be discussed.

L-20
DECHLORANES: THE NEXT EMERGING POPS?

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Dechloranes such as Dechlorane Plus (DP, $C_{18}H_{12}Cl_{12}$), Dechlorane 602 (Dec 602, $C_{14}H_4Cl_{12}O$), Dechlorane 603 (Dec 603, $C_{17}H_8Cl_{12}$), Dechlorane 604 (Dec 604, $C_{13}H_4Br_4Cl_6$) and Chlordene Plus (CP, $C_{15}H_6Cl_{12}$) possess flame retardant properties similar to Mirex. Whereas the use of DP as a flame retardant (electrical wires, cables coating, computers and polymers) is well established, little information is available for the use of the other dechloranes. All these compounds are unregulated compounds and represent a possible alternative to other regulated FRs such as the polybrominated diphenyl ethers (PBDEs).
The environmental occurrence of dechloranes was first reported in 2006 in North America when DP was detected in air, sediment and fish samples from the Laurentian Great Lakes (Hoh et al., 2006). In 2010, other dechloranes such as Dec 602, 603 and 604 were reported in sediment and fish samples from the same area (Shen et al., 2010). CP was later detected in sediments (Shen et al., 2011). Even though the number of studies is still small, additional data collected in Korea, Brazil, North Africa, Spain and Germany (de la Torre et al., 2012; de la Torre et al., 2011; Kang et al., 2010; Munoz-Arnanz et al., 2012; Sühring et al., 2013) indicate that DP and related compounds should be considered as possible worldwide contaminants.
Two recent review papers described sources, occurrence and behavior of dechloranes in the environment, concluding on the need of more research dedicated to the production of data on exposure and toxicity (Sverko et al., 2011; Xian et al., 2011). Additionally to the fact of considering environmental contamination and geographical distribution, a better understanding of the behavior of DP and related compounds in terms of bioaccumulation and biomagnification is still needed. Even more importantly, virtually no biological monitoring (biomonitoring) data are available for any of the dechloranes.
The present study had two main objectives. The first was to develop an analytical procedure to isolate and measure levels of DP, Dec 602, Dec 603, Dec 604, and CP. The second was to measure levels of these compounds in human serum samples in a selected part of the Western Europe population and compare them to PBDE levels to estimate the significance of dechlorane levels.
As dechlorane levels appeared to be comparable to PBDE levels, it is now important to further investigate what the route of exposure are and if our food web has to be monitored for such emerging compounds.

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L-21*
VALIDATION OF GC-MS/MS CONFIRMATORY METHOD FOR THE EU OFFICIAL CONTROL OF LEVELS OF PCDDS AND DL-PCBS IN FEED MATERIAL OF PLANT ORIGIN

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Criteria for sampling and analysis for the official control of levels of dioxins (including polychlorinated dibenzo-*p*-dioxins -PCDDs- and polychlorinated dibenzofurans -PCDFs-), and dioxin-like PCBs (DL-PCBs) in feeding stuff and in certain foodstuffs are described in Commission Regulations (EU) No 278/2012 and 252/2012[1,2]. They require the application of gas chromatography coupled with high resolution mass spectrometry (GC–HRMS) as confirmatory method for the unequivocal identification and quantification of PCDD/Fs and DL-PCBs at the level of interest.
In 2011, based on recent reports about performances of new generation tandem mass spectrometry (MS/MS) systems[3-5], a specific core working group was formed within the network of European Union Reference Laboratory (EU-RL) and National Reference Laboratories (NRLs) of EU Member States to investigate the capability of GC coupled to MS/MS (mainly triple quadrupole analyzers) based methods for potential use as an alternative confirmatory method for dioxins and PCBs in Feed and Food[6]. After amendments of the current criteria for confirmatory methods, it was concluded that GC–MS/MS systems could possibly be used as an alternative to GC–HRMS systems as confirmatory methods.
The present study reports on the first validation of a GC-MS/MS method for quantification of PCDD/Fs and DL-PCBs in feed materials of plant origin (vegetable oils) at the regulation level[7] of 0.75 ng WHO-PCDD/F-TEQ/kg and 1.5 ng WHO-PCDD/F-PCB-TEQ/kg. Results are further compared to the current routine confirmatory GC-HRMS method.

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[7] Commission Regulation (EU) No 277/2012 of 28 March 2012 amending Annexes I and II to Directive 2002/32/EC (OJ L 91, 29.3.2012, p. 1–7)

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L-22*
POTENTIAL OF GAS CHROMATOGRAPHY-(TRIPLE QUADRUPOLE) MASS SPECTROMETRY COUPLED TO ATMOSPHERIC PRESSURE CHEMICAL IONIZATION FOR POP ANALYSIS

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Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and several pesticides are contemplated in the international Stockholm Convention on Persistent Organic Pollutants (POPs) among the twelve most toxic chemical substances. Their persistent character and physical chemical characteristics favors the bioaccumulation in the fatty tissues and, consequently, the biomagnification in the food chain, leading to a potential significant impact on human health, even at trace levels. The use of gas chromatography (GC) coupled to mass spectrometry (MS) with electron ionization (EI) has been widely used for the determination of these contaminants in food commodities over the past decades. However, in typical GC–(EI) MS/MS methods operating under Selected Reaction Monitoring (SRM), transitions used are rarely based on fragmentation of the molecular ion (M+•), which means that a loss of sensitivity can occur and that the specificity of the method can be also compromised. This makes that the essence and potential advantages of tandem MS are, in some way, lost. On the contrary, the new available atmospheric pressure chemical ionization (APCI) source produces a softer ionization that reveals abundant molecular ions in the mass spectra, both as M•+ or as [M+H]+. Thus transitions in SRM methods can include the molecular or quasi molecular ions as precursors, which results in an enhancement of sensitivity and selectivity comparing to the conventional methods based on EI, as has been demonstrated in this work. The use of a GC–(APCI) MS/MS with a triple quadrupole analyzer has allowed the development and the successful validation of different methodologies for the determination of several POPs including dioxins, dioxin-like PCBs and pesticides in food commodities. On one hand, the high sensitivity obtained for the PCDDs/Fs and PCBs led to results comparable to those obtained using GC coupled to high resolution mass spectrometry with magnetic sector analyzer, which is considered the reference method for the determination of dioxins and related compounds. On the other hand, regarding to the pesticides field, some problematic cases due to the absence of specific SRM transitions in GC–(EI) MS/MS methods could be overcome taking profit of the high selectivity provided by this new system. Moreover, the high sensitivity allowed simplifying the sample treatment by diluting the sample extract by a factor of 10, which reduces matrix effects at the same time. Results are optimistic for this new approach in food safety for future improvements of multi-residue quantitative analysis, involving cheaper and less complex instrumentation.

Keywords: *Atmospheric pressure chemical ionization, gas chromatography, tandem mass spectrometry, persistent organic pollutants*

L-23
COMPARISON OF VARIOUS TANDEM GC–MS TECHNIQUES IN ANALYSIS OF HALOGENATED POPs IN FISH (QTOF VERSUS QQQ)

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Fish is considered to be an irreplaceable component of human diet representing an important source of long chain n-3 unsaturated fatty acids, iodine, selenium and vitamins A and D; however at the same time may significantly contribute to dietary exposure to various contaminants including e.g. persistent organic pollutants (POPs). Even though these chemicals are commonly present at (ultra)trace concentrations (μg/kg), long-term exposure may trigger various adverse health effects, therefore needed to be monitor. Both usually extremely low concentrations in fish muscle tissue and the complexity of such a matrix, make this analysis somehow difficult. Gas chromatography (GC) coupled to tandem mass spectrometry (MS/MS) represents a powerful tool for highly sensitive and selective determination of various groups of environmental contaminants including polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), organochlorinated pesticides (OCPs) and poly cyclic aromatic hydrocarbons (PAHs). In the presented study, the application potential and suitability of GC–MS/MS with a quadrupole–time of flight (QTOF) mass analyzer for the (ultra)trace analysis of POPs and PAHs in fish tissue was evaluated and compared to GC–MS/MS employing a triple quadrupole (QqQ) mass analyzer which has already been successfully implemented in the analysis of POPs and PAHs in our previous studies. In general, MS/MS allows one to minimize matrix component interferences, and at the same time, thanks to the possibility of selecting suitable precursor and product ions, makes possible identification and quantification of the above-mentioned contaminants even at (ultra)trace concentrations. Additionally to QqQ, the QTOF mass analyzer provides accurate mass information due to which molecular characterization and structural confirmation both in target and non-target analysis could be done. In the first part of our experiments, a GC–QTOF–MS/MS method was developed and optimized to obtain low limits of quantification (LOQs) and in the case of isomeric compounds, which cannot be resolved based on an accurate mass, also high chromatographic resolution. Subsequently, under the optimized GC–MS/MS conditions, a batch of real life marine fish samples was analyzed using both QqQ and QTOF to evaluate and compare their performance and applicability. The examined samples included five fish species (Horse mackerel, Red fish, Red mullet, Gilthead seabream and Sea bass) of a different geographic origin (Black Sea, Marmara Sea and Aegean Sea). In total, all samples tested were 'positive', containing at least 18 target analytes; however, no sample exceeded the limits set in EU legislation for sum of dioxin-like PCBs and sum of major PCBs in fresh fish meat. CB 153 and p,p'-DDE were the most frequently found contaminants. Regarding contamination of fish species, the levels of pollutants decreased in the following order: Sea bass > Red mullet > Gilthed seabream ~ Horse mackerel > Red fish.

Keywords: *GC–QQQ–MS/MS, GC–QTOF–MS/MS, environmental contaminants, fish*
Acknowledgement: *Financial Support from Specific University Research (MSMT No. 20/2013).*

L-24
ANALYSIS OF PHOSPHORUS BASED FLAME RETARDANTS – ANOTHER GROUP OF BIOACCUMULATING CONTAMINANTS

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Due to a ban on several brominated flame retardants such as pentabromodiphenylethers, industries have increased the production of organophosphorus flame retardant (PFR) over recent years. The percentage PFR production of the total worldwide flame retardant production volume has increased from 14% in 2004 to 19% in 2010. In many products such as furniture, textile, cables, building material, insulation materials, paint, floor polish, hydraulic fluids and electronic appliances, PFRs are also used as plasticizers. In most applications PFRs are used as additive chemicals and thus not covalently bound to the polymeric materials. During use or after their disposal PFRs can leach into the environment. Neurotoxic effects have been observed for tricresyl phosphate (TCP), triphenyl phosphate (TPP) and tributyl phosphate TBP and the chlorinated PFRs tris (1,3-dichloropropyl-2) phosphate (TDCPP), tris 1-chloro-2-propyl phosphate (TCPP) and tris (2-chloroethyl) phosphate (TCEP) are carcinogenic for animals. The first environmental concentrations of PFRs were already reported in the late 1970s. More recently, high concentrations of PFRs were observed in house dust from different countries. The most abundant PFRs observed in dust are tri-isobutyl phosphate (TiBP), tris 2-butoxyethyl phosphate (TBEP), TCPP and TPP, in the 1–20 μg/g ranges. PFRs have also been detected in outdoor environmental compartments, such as river water, groundwater, wastewater and sediment. PFRs show strongly different chemical and physical properties. The log K_{ow} values vary from 3.6 for TiBP to 9.49 for TEHP. Therefore, some PFRs dominate in air or water and others adsorb stronger to particle mater. Chlorinated PFRs are more resistant to biodegradation than the alkyl and aryl phosphates. Limited information is available on PFR concentrations in biota. The focus of the present study was to develop a fast analytical method to analyze twelve PFRs with LC–MS/MS in biota and sediment samples. Furthermore, the bioaccumulation of PFRs in two food webs of the Western Scheldt (the Netherlands) was studied. Analysis of PFRs was performed on a 1260 infinity HPLC (Agilent Technologies) with a 150 × 3 mm Luna C18 (3 μm) column (Phenomenex), coupled to a 6410 triple quad MS (Agilent) with an electro- spray ionization (ESI) interface measuring in the positive mode. The extracts were also analyzed by GC–electron impact–MS. However, due matrix interferences LC–MS/MS was selected as the more robust technique for the analysis of PFRs in biota and sediment. Good recoveries were obtained for all PFRs analyzed in sediment, fish oil and sole. An international interlaboratory study showed that, due to background contamination from dust can contain relatively high levels of various PFRs (up to 100 ng/g). TCPP, TCEP and TBEP are accumulating in aquatic food webs, and also TiBP was observed in fish. Human exposure will be both from food and house dust.

Keywords: *Organophosphorus flame retardants, LC–MS, food chain, interlaboratory study*

L-25
EFSA CONTAM PANEL: HOW IT CARRIES OUT RISK ASSESSMENTS ON CONTAMINANTS

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European Food Safety Authority (EFSA)* carries out risk assessments on food and feed safety at the European level. In the European food safety system, risk assessment is done independently from risk management. As the risk assessor, EFSA produces scientific opinions and advice to provide a sound foundation for European policies and legislation. Thus EFSA supports the European Commission, European Parliament and European Union Member States in their risk management decisions. EFSA's remit is wide and covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. In the process of developing its scientific opinions, EFSA's Scientific Panels and Committee have crucial roles. The experts of the Scientific Panels and Committee from all over Europe and the world contribute to the scientific opinions. The EFSA Panel on Contaminants in the food chain (CONTAM Panel)** carries out risk assessments in the area of chemical contaminants in food and feed, namely process contaminants, environmental contaminants, natural toxicants, mycotoxins and residues of unauthorised substances. In order to assess the risk for public and/or animal health and to prepare the related scientific opinions, the CONTAM Panel collects and scrutinises scientific information available in the public domain on the contaminants, their occurrence in food and feed, exposure to humans and animals, toxicokinetics and toxicity including dose-response data. Within this risk assessment process, the CONTAM Panel establishes health based guidance values for various contaminants, c ompares the estimated exposure levels to the established health based guidance values (humans), or to the identified no-observed-adverse-effect levels (animals). The CONTAM Panel can also calculate margin of exposure and finally it concludes on the risk for humans and/or animals. The CONTAM Panel scientific opinions then advice and help the risk managers such as the European Commission and Member States to decide the need for possible revisions of the current legislation and/or any other possible follow-up actions required in relation to contaminants in food and feed. This presentation gives an overview how the EFSA CONTAM Panel carries out risk assessment on contaminants in food and feed.

* http://www.efsa.europa.eu/
** http://www.efsa.europa.eu/en/panels/contam.htm

Keywords: *EFSA, risk assessment, contaminants*

L-26
COLLATION OF CHEMICAL OCCURRENCE DATA FOR EXPOSURE ASSESSMENT

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Assessment of exposure is a central step in any risk assessment of substances whose presence in food can lead to adverse health effects. Dietary exposure to potentially hazardous substances is usually assessed by combining measured chemical occurrence levels in relevant food products with detailed data on their consumption in a population. EFSA doesn't generate any occurrence data for European risk assessments, but collates existing data throughout Europe. A call is open on a permanent basis for a defined list of persistent and organic pollutants, heavy metals, mycotoxins, plant toxins, processed contaminants and pesticide residues. Ad hoc calls are also launched according to specific needs of the some risk assessments. In order to guarantee a good understanding of the information and to facilitate further data handling, EFSA has developed a standard for chemical occurrence data exchange. The EFSA Standard Sample Description (SSD) defines a list of 76 elements which allows to fully describe a food sample, how it has been taken, the chemical measured, the analytical method used, the result obtained and its uncertainty. Most of these elements follow a controlled terminology. In particular, the food which must be described according to the EFSA food classification and description system for exposure assessment (FOODEX). The SSD is generic and allows to handle all kinds of chemical and microbiological hazards. However, all the data elements are not adapted nor required for all compounds. Around 20 elements are mandatory in all cases, the other being mandatory or recommended on a case-by-case basis. For example, the indication of fat content is required only for compounds which are measured after the extraction of the fat, such as dioxins. Additional requirements can also be applied on the analytical side in order to optimise quality and comparability of the results. Most of them follow the analytical requirements defined in the European legislation for the data collected in the framework of official monitoring programmes. Overall, the laboratories must be accredited and the analytical method validated for the compound of interest. According to the compound, the analytical technique to be used, the range of acceptable recoveries or the maximum acceptable limit of quantification by food matrix can also be specified. Finally, besides the analytical requirements, it is also important that the occurrence data are representative of the levels found in foods as consumed by the European population. Improvements are foreseen in this direction with the development and harmonisation of the Total Diet Study (TDS) approach. TDS are surveys designed to determine population dietary exposure to chemical substances across the entire diet by analysing main foods prepared as consumed and pooled into representative food groups.

Keywords: *Occurrence data, Standard Sample Description, analytical requirements, Total Diet Study*

L-27
EFSA CONTAM PANEL RISK ASSESSMENTS OF HEAVY METALS – THE EXAMPLE OF MERCURY AND METHYLMERCURY IN FOOD

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The European Food Safety Authority (EFSA) has been asked by the European Commission to assess the risks to human or animal health related to the presence of some specific heavy metals in food or feed during the past years. EFSA has published since 2002 risk assessments on lead, cadmium, mercury and inorganic mercury and arsenic in food and on lead, cadmium, arsenic and mercury as undesirable substance in animal feed. Recently, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to assess human dietary exposure to inorganic mercury and methylmercury and to consider new developments regarding the toxicity of inorganic mercury and methylmercury and to evaluate whether the provisional tolerable weekly intakes (PTWIs) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of 1.6 µg/kg body weight (b.w.) for methylmercury and of 4 µg/kg b.w. for inorganic mercury were considered appropriate. In line with JECFA, the CONTAM Panel established a tolerable weekly intake (TWI) for inorganic mercury of 4 µg/kg b.w., expressed as mercury. For methylmercury, new developments in epidemiological studies from the Seychelles Child Developmental Study Nutrition Cohort have indicated that n-3 long-chain polyunsaturated fatty acids in fish may counteract negative effects from methylmercury exposure. Together with the information that beneficial nutrients in fish may have confounded previous adverse outcomes in child cohort studies from the Faroe Islands, the Panel established a TWI for methylmercury of 1.3 µg/kg b.w., expressed as mercury. Following a call for annual collection of chemical contaminant occurrence data in food and feed, including mercury, EFSA received 59,820 results on mercury in food from 20 European countries, mainly covering the period from 2004 to 2011. The majority of the samples (98.2%) were for total mercury, 1.8% for methylmercury and three samples for inorganic mercury. The mean dietary exposure across age groups does not exceed the TWI for methylmercury, with the exception of toddlers and other children in some surveys. The 95th percentile dietary exposure is close to or above the TWI for all age groups. High fish consumers, which might include pregnant women, may exceed the TWI by up to approximately six-fold. Unborn children constitute the most vulnerable group. Biomonitoring data from blood and hair indicate that methylmercury exposure is generally below the TWI in Europe, but higher levels are also observed. Exposure to methylmercury above the TWI is of concern. If measures to reduce methylmercury exposure are considered, the potential beneficial effects of fish consumption should also be taken into account. Dietary inorganic mercury exposure in Europe does not exceed the TWI, but inhalation exposure of elemental mercury from dental amalgam is likely to increase the internal inorganic mercury exposure; thus the TWI might be exceeded.

Keywords: *Heavy metals, risk assessment, food, methylmercury, inorganic mercury*

Acknowledgement: *EFSA wishes to thank the members of the former EFSA WG on Mercury in food and the former and current members of the Panel on Contaminants in the Food Chain (CONTAM Panel, http://www.efsa.europa.eu/en/contam/contammembers.htm). EFSA and the CONTAM Panel acknowledge all the European countries that provided mercury occurrence data for food and supported the consumption data collection for the Comprehensive European Food Consumption Database.*

L-28
EFSA CONTAM PANEL RISK ASSESSMENTS OF BROMINATED FLAME RETARDANTS (BFRs) IN FOOD

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The European Commission had requested EFSA to assess the risks to human health related to the presence of brominated flame retardants (BFRs) in food, considering the following classes of BFRs in six different scientific opinions: *(i)* polybrominated biphenyls (PBBs), *(ii)* polybrominated diphenyl ethers (PBDEs), *(iii)* hexabromocyclododecanes (HBCDDs), (iv) tetrabromobisphenol A (TBBPA) and its derivatives, *(v)* brominated phenols and their derivatives and *(vi)* emerging and novel BFRs. EFSA was asked to consider all relevant toxicological information and to carry out a dietary exposure assessment for the general population and specific groups of the population. Biomonitoring data for these compounds should also be taken into account, and potential data gaps for the BFRs considered should be identified. This task was allocated to the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) and a Working Group was established for this purpose. The last opinion of this series, on Emerging and Novel BFRs, was published September 2012. All opinions are published in the EFSA Journal and are available through the EFSA website (www.efsa.europa.eu). The assessment of the risk was done following the risk assessment paradigm: hazard identification, exposure assessment, hazard characterization and risk characterization. The exposure assessment combined the data on human consumption available for the different food categories using the EFSA Comprehensive European Food Consumption Database, with the occurrence data on BFRs in the respective food categories. A range of intake/exposure scenario estimates were considered so that special subgroups of the population (e.g. infants and children, vegetarians) and high consumers, were covered. For the purpose of these series of opinions on BFRs, the occurrence data from the EU-wide BFR monitoring recommended by the EC in 2006 was made available to EFSA. In addition, to ensure that human exposure assessment was as comprehensive as possible, EFSA launched a call for data in 2009 (closed by end 2010). For the hazard characterization, available toxicological, toxicokinetic and epidemiological studies in the open literature until the publication of the opinions were considered. Due to the limitations and uncertainties in the toxicological database for the BFRs under evaluation, the derivation of health based guidance values (e.g. TDI) was not considered appropriate, and instead the margin of exposure (MOE) approach for the risk characterization was used, when possible. For the novel and emerging BFRs considered, and due to the very limited available information on occurrence, exposure and with respect to their toxicological hazards, it was not possible to perform a risk characterisation. However, the CONTAM Panel made an attempt to identify those emerging or novel BFRs that could be a potential health concern and should be considered first for future investigations

Keywords: *Brominated flame retardants, risk assessment, food, human exposure, toxicity*

Acknowledgement: *EFSA wishes to thank the members of the former EFSA WG on BFRs and the former and current members of the Panel on Contaminants in the Food Chain (CONTAM Panel, http://www.efsa.europa.eu/en/contam/contammembers.htm). EFSA and the CONTAM Panel acknowledge all the European countries that provided BFR occurrence data in food and supported the consumption data collection for the Comprehensive European Food Consumption Database*

L-29
RISK ASSESSMENTS ON MYCOTOXINS IN FOOD AND FEED – SCIENTIFIC OPINIONS OF THE EFSA CONTAM PANEL

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European Food Safety Authority (EFSA) does risk assessments on food and feed safety at the European level. As the risk assessor, EFSA produces scientific opinions and advice to provide a sound foundation for European policies and legislation. Thus EFSA supports the European Commission (EC), European Parliament and EU Member States in their risk management decisions. The remit of EFSA covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. In the process of developing its scientific opinions, EFSA Scientific Panels and Committee have crucial roles. The EFSA Panel on Contaminants in the food chain (CONTAM Panel) carries out risk assessments in the area of chemical contaminants in food and feed, namely process and environmental contaminants, natural toxicants, mycotoxins and residues of unauthorised substances. This presentation outlines the CONTAM Panel's recently published scientific opinions on the risks to human and/or animal health related to the presence of mycotoxins in food and/or feed. The mycotoxins covered were zearalenone, Alternaria toxins, T-2 and HT-2 toxins, nivalenol, phomopsins, citrinin, sterigmatocystin and ergot alkaloids*. These risk assessments were all developed upon requests from the EC. To address the specific areas identified in the mandates from the EC, the CONTAM Panel set up various working groups comprising scientists with appropriate expertise from the European or international research institutions to prepare the scientific opinions on mycotoxins. The main outcomes of the aforementioned scientific opinions are presented. In addition, the future activities of the CONTAM Panel in relation to mycotoxins in food and feed will be outlined.

* http://www.efsa.europa.eu/en/panels/contam.htm

Keywords: *Risk assessment, mycotoxin, food, feed*

Acknowledgement: *The members of the EFSA CONTAM Panel and the members of the CONTAM Working Groups (WG) on Zearalenone, Alternaria toxins, Fusarium toxins, Phomopsins, Aspergillus toxins and Alkaloids.*

L-30
THE HORSEMEAT INCIDENT: IMPLICATIONS FOR FUTURE STRATEGIES IN FOOD ANALYSIS

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On 15 January 2013 the Food Safety Authority of Ireland reported data on a surveillance study on the speciation of meat products. Included in the study were 27 beef burger products, 10 of which tested positive for horse DNA. These results had far reaching consequences for the European food industry and regulatory authorities as subsequent testing demonstrated that this was not a localised problem but affected many parts of Europe. The resulting loss of consumer confidence in the meat industry and retailers has had major implications for the companies caught up in the incident and for the European food supply in general. In particular it has elicited a major reappraisal of the need for food testing; identified the need for better understanding and monitoring of food supply chains, as well as a great deal of debate about where food fraud sits within what is largely at present, a food safety dominated regulatory framework. The incident also emphasised the lack of a formal network of expertise that stakeholders could call on for advice and expertise in food authenticity. This was emphasised in the considerable confusion about the interpretation of the results of DNA analysis and identified the need for quantitative methods and multi-species methodology. On a larger scale the incident has transformed the food chain agenda. There is now a focus on demonstrating 'food integrity' to the consumer with concomitant transparent assurance of food safety, quality and authenticity attributes. It is clear that the burden of this assurance will fall on the food industry. In the short term this means an increase in testing but it is unclear how this can be sustained in its present form. New analytical strategies are needed that can provide more cost effective verification measures for the food industry. The challenges posed to food analysts will be discussed together with possible solutions that could provide step changes in the way we analyse our food.

Keywords: *Food safety, food authenticity, food analysis, food integrity, food assurance*

L-31
MULTI-ELEMENT ANALYSIS BY ICP-OA-TOF-MS FOR TRACING THE GEOGRAPHICAL ORIGIN OF TOMATO PRODUCTS

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For the last few years, consumers in the Mediterranean area have shown a keen interest in food "authenticity", so many analytical methods have been developed to trace reliably the geographical provenance of food. The elemental composition of a vegetable-based food can be used as a marker for its origin, given the close chemical link between vegetables and the soil where they were grown. In this work we evaluated the analytical potential of Inductively-Coupled-Plasma orthogonal-acceleration Time-Of-Flight Mass Spectrometry (ICP–oa–MS–TOF), using the GBC Optimass 9500 spectrometer, to implement a multi-element, database-comparison technique for the determination of geographical origin. As an example, we studied the tomato, a food matrix of special interest in the Mediterranean. For several samples of various tomato products (purée, double and triple concentrate paste) acquired on the market, provided by companies, or produced in the SSICA pilot plants in the 2012 tomato campaign, we used mass spectrometry to determine the concentration of rare earths (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) and of 23 other elements (Li, Be, Al, V, Cr, Mn, Co, Cu, Zn, Ga, As, Rb, Sr, Mo, Ag, Cd, In, Cs, Ba, Tl, Pb, Bi, and U). The geographical origin (Italy, Spain, or China) of each sample was known. We experimented with different wet procedures for mineralization: acid mixtures with different oxidation-reagent combinations (HNO_3, HCl, H_20_2, $HClO_4$), and digestion either in disposable closed vessels on a hot plate at 100°C (using SCP Science Digiprep®) or in a microwave oven at 200°C (using CEM Mars® Xpress). We finally adopted an optimized procedure using HNO_3/H_20_2 and closed-vessel digestion. To correct for matrix effects and instrumental drifts across the spectrum, we added three internal standards (Be, In, and Bi) to the calibration solutions and to the samples. The instrumental detection limits (LODs) ranged from 0.001 µg/kg for Pr, Tb, Ho, Tm, and Lu, to 0.68 µg/kg for As. Our statistical analysis, carried out for specific ratios of metal isotopes, showed a high discrimination power for certain elements. By contrast, rare earths, which are present in tomato at low concentrations ranging from 0.15 µg/kg dry weight for Tm at Lu to 35.2 µg/kg dry weight for Ce, cannot be used as discriminant markers of geographical origin.

Keywords: *Authenticity, geographical origin, rare earths, ICP-TOF-MS, tomato*

Acknowledgement: *The authors are grateful to Fondazione Cariparma for financial support to SSICA, under P1218 project.*

L-32
DISCRIMINATION OF ORGANIC FOODS FROM OTHERS FARMING'S TYPE USING RDNA FINGERPRINTING OF MICROBIAL COMMUNITIES BY PCR-DGGE: AN APPLICATION ON NECTARINES FRUITS.

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Since 2005, the European regulation 178/2002 imposes the traceability process of foodstuffs (Article 17). In practice, traceability of foods is mainly done at the administrative level, and the use of analytical tools is rare. Previous studies have demonstrated that microbial ecology analyses at the molecular level (such as PCR–DGGE) could be used to provide food with a unique biological signature that could be linked to the geographical origin of food. The aim of this project was to use this approach to test whether we could differentiate organic from conventional foods, in order to improve the traceability of such products. The hypothesis was that the different processes applied to different types of agriculture have an influence on the microorganisms that are present on foods. In our study, analysis of yeast and bacterial rDNA DGGE profiles revealed that yeast and bacteria communities were specific of organic nectarines and could discriminate organic fruits from conventional or sustainable fruits. We showed also that microbial ecology structure (yeast and bacteria) is specific of the production mode. Some species, well identified could be used as biological markers to certify the origin as well as the mode of production of foodstuff. We proposed this analytical tool as a first step to control and authentify organic food.

Keywords: *Traceability; biological bar code; organic foods; food microbiology; PCR-DGGE.*

L-33*
UTILIZATION OF METABOLOMICS FOR SAFFRON AUTHENTICATION

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Saffron (*Crocus sativus* L.) is a traditional, highly valuable spice due to its coloring properties, alluring aroma and pleasant bitter taste. Since saffron is the most expensive spice in the world, it is susceptible to adulteration like addition of other plant materials or artificial colorants. A wide range of analytical approaches is currently used for saffron authentication. Non-target screening of compositional characteristics represents a promising alternative to the traditional analytical techniques, which are mostly limited to a few analytes or classes of analytes. In this presentation, the potential of metabolomics as a challenging tool for the assessment of saffron authenticity is demonstrated. For this purpose, two different strategies were tested: ultra-high performance liquid chromatography coupled to quadrupole – time of flight mass spectrometer (UHPLC–QTOFMS) and ambient mass spectrometry employing a Direct Analysis in Real Time ion source coupled to high resolution Orbitrap mass spectrometer. Within this study, a wide set of saffron samples (overall 56 samples from Czech and Spanish market) were analyzed. Data measured using both above mentioned techniques were treated using an unsupervised pattern recognition technique: Principal component analysis (PCA). From the obtained PCA scores plot, some clusters of the samples were evident. For example, The PCA of metabolomic fingerprints revealed clear differences between saffron cultivated in Spain (Protected Designation of Origin, La Mancha and Aragon) and saffron packaged in Spain, showing that picrocrocin, crocin, crocetin and their glucosides were important markers. Also some outliers were observed in the PCA scores plot; they had opposite position in the scores plot than the origin protected samples. It was observed that these samples had lower intensities of the saffron typical markers and, therefore, they were probably adulterated. At the end, the retrospective UHPLC–HRMS data analysis allowed organic and natural dyes to be identified in commercial saffron. Using UHPLC–MS, even three artificial colorants were detected in one sample. After the exclusion of the outliers (probably adultered samples), it was also possible to differentiate between saffron cultivated in Spain and saffron packaged in Spain, as well as the origin of saffron.

Keywords: *Saffron, authenticity, UHPLC–MS, DART–MS, fingerprinting/profiling*

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L-34
ARTIFICIAL INTELLIGENCE APPLIED ON COMPLEX DATA SET FROM CHEMOTYPING AND GENOTYPING OF FOODS: APPLICATION TO AUTHENTICATION AND TRACEABILITY. HAZELNUT, LUPIN AND RICE CASE STUDIES

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As showed by many researches in the last decade, the chemotyping and genotyping comprehensive approach may be useful both for the authentication and traceability of foods and food ingredients. This complex (and sometimes time/money-consuming) approach often leads to obtain big complex data sets difficult to manage using classical statistical methods. Intelligent analysis of data is, beside the strategy devoted to select the more significant and descriptive parameter is a key strategy in order to recognise/trace a food in a inexpensive way. The so-called "data mining" (complex statistical and mathematical treatment of the data sets, often exploiting Artificial Intelligence-based algorithms) - particularly the "hierarchical" and "clustering" studies – is largely exploited by food scientists, in order to decrease complexity and to obtain "readable comprehensive" outcomes. Principal Component Analysis is a common example of this kind of approach, but other specific and performing strategies (e.g. Artificial Neural Networks, like Self Organizing Maps or Back Propagation, Bayesians Networks, Genetic Algorithms and others) are of great interest in this field. The Aim of this communication is focused to show some examples of "complex data set management". The complex data sets, obtained both from chemotyping (chromatographic, electrophoretic, spectrophotometric methods and other chemical/analytical approaches) and genotyping characterization (Polymerase Chain Reaction-based techniques, like RAPD, SNP's) of hazelnut (*Corylus avellana*), lupin (*Lupinus albus* and *L. angustifolius*) and rice (*Oryza sativa*) from different geographical origins/varieties/cultivars were processed using different statistical approaches, based on Artificial Intelligence. Advantages and critical aspects will be discussed here, showing that exploiting the comprehensive processing of "omics" (metabolomic, genomic) data leads to the solving of different classification/identification/ authentication problems, often not solved from the statistical processing of a single data set [1,2].

[1] The artificial based chemometrical characterisation of genotype/chemotype of Lupinus albus and Lupinus angustifolius permits their identification and potentially their traceability Food Chem, 129, 4, 1806–1812 (2011)
[2] Chemotype and genotype chemometrical evaluation applied to authentication and traceability of "Tonda Gentile Trilobata" hazelnuts from Piedmont (Italy), Food Chem, 129, 4, 1865–1873 (2011)

Keywords: *Authentication and traceability, Intelligent Analysis of Data, genotyping, chemotyping*

L-35
ARE YOU SURE YOU'RE DRINKING A CABERNET SAUVIGNON?

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The wine sector is one of the economically most important agriculture activities in the World. Specific *Vitis vinifera* L. varieties are considered as the key factors for Premier wines, giving them an added value related to their enological characteristics. However, under legislation the inclusion of other varieties in defined percentages is permitted. This procedure attracts fraudulent practice sometimes difficult to track. Therefore, a tight traceability system able to identify grapevine varieties is needed throughout the wine making process, from the vineyard until the bottled wine. Several compounds have been considered for this purpose (phenolic and volatile compounds, among others), however, the DNA seems to be the most reliable for varietal identification, once it is not dependent on any external factor. Grapevine genotyping is currently based on microsatellite markers or simple sequence repeats (SSR), which have been very useful for genetic identification. Six nuclear SSR were previously accepted as universal markers for grapevine genotyping (European GENRES-081 research project). After the establishment of a viable DNA extraction protocol from musts and wines, in our research group, and by allelic size determination it was possible to find an unequivocal correspondence between grapes, musts, and wines. Nevertheless, in the last few years, single nucleotide polymorphisms (SNPs) have become the most popular genetic marker system in animals and plants. Their higher abundance combined with recent technological improvements makes SNP markers an excellent tool for grapevine genotyping. SSCP analysis detects sequence variations (single-point mutations and other small-scale changes) through electrophoretic mobility differences. High resolution melting curve (HRM) is a recent advance technique for the detection of SNPs and INDELs in PCR amplicons and depends on DNA melting in the presence of saturating intercalating double-stranded DNA binding dyes. Anthocyanins represent a major group of the flavonoid family, with a crucial role in wine colour and its organoleptic properties. The aim of the study was to identify SNP markers in selected grapevine varieties (red and white) concerning UFGT gene coding for UDP glucose–flavonoid 3-O-glucosyl-transferase, involved in anthocyanin biosynthesis. In silico analysis of UFGT gene sequence was performed and primer pairs were designed using Primer3 software. PCR amplicons were sequenced and SNPs were annotated. An assay was designed to rapidly detect SNPs using both SSCP and HRM approaches, allowing the differentiation of the grapevine varieties according to their sequence variants. The establishment of an efficient traceability system is important so that consumers trust wine labeling. The use of DNA fingerprinting will provide tools for that purpose through grapevine varietal identification during vinification process, starting with the selection of the grapes and ending with bottled wine.

Keywords: *Traceability, grapevine identification, SSR, anthocyanins, SNPs*

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L-36
RAPID STRATEGY FOR SPIRITS QUALITY AND SAFETY CONTROL

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Substituting genuine beverage by an article made from rectified spirit saves a great deal of time and expenses by its aging and storage. Alcoholic beverages contain higher alcohols, aldehydes, esters, acids, and other compounds creating the flavor and characteristics of the particular product. Lower than expected methanol and higher alcohol concentrations may indicate non-authentic product made in part with (low congener) neutral alcohol. High methanol concentrations may indicate the presence of non-authentic product made from an industrial alcohol that may be potentially harmful if consumed. Last year, when spirits in the Czech Republic were extensively adulterated with methanol, more than 40 people died in consequence. Therefore, rapid analytical strategy for the reliable assessment of quality and authenticity of spirits in "low-volume" samples are needed. Official reference methods for the analysis of spirits drinks are described in the Commission regulation (EC) No 2870/2000. Alcoholic strength is determined (after distillation of sample) by either pycnometry or densimetry; gas chromatography (GC–FID) is used for analysis of volatiles/congeners (methanol, acetaldehyde, higher alcohols, etc.). Nevertheless, these procedures are time consuming and high volumes of spirits sample are needed. Although gas chromatography coupled to mass spectrometry (GC–MS) is more selective than routine method, the application of GC–MS using isotopic dilution for the spirits drinks analysis was not reported yet. Sample preparation for this new GC–MS analysis is very fast, requires only 50 µl of sample and addition of isotopically labeled internal standards (ethanol-2,2,2-d_3, and methanol-d_3). GC coupled to a single quadrupole MS was used for analysis of both methanol, ethanol and other compounds. Validation was carried out on samples with different content of ethanol and other volatiles (pear brandy, grappa, caribbean rum, whisky and vodka). Repeatability expressed as a relative standard deviation varies in range from 0.3% to 0.5 % at six replicates for most of congeners. Also commonly used spirit denaturants, mainly 2-methyl-propan-2-ol and propan-2-ol, can be determined (LOQ ≤ 0.2 g/hl a.a.). Successful participation in proficiency testing (FAPAS report 1368: alcohol and congeners in whisky, 2013) confirmed the suitability of this method for rapid evaluation of spirits drinks quality in both routine beverage control and forensic examination of suspected liquid residues.

Keywords: *spirits; volatile compounds; methanol; isotopic dilution; GC–MS*

Acknowledgement: *Financial support from specific university research (MSMT No 20/2013)*

L-37
THE CHEMISTRY OF FOOD FLAVOURS: SIMPLY PLEASURE OR BEYOND?

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During eating, a distinct set of food constituents induces a pattern of neural activity in the human olfactory and gustatory systems. However, we all know by experience that the complex neural patterns generated at the receptor sites on the tongue and in the nose are finally “translated” by our brain into a simple perception telling us the overallflavor quality of a food. However, although thousands of food constituents have already been identified, only a few studies have attempted to clarify the chemical background of food flavor perception. The concept of Sensomics, developed by our group, allows one to decode the blueprint of those genuine key flavor compounds able to interact with the odorant receptors during food consumption. This interaction with the peripheral receptors renders bioactivity to odorants as well as to tastants. However, interestingly, for certain food constituents, besides flavor properties, other “bioactivities” have been repor ted, such as effects on behavior, mood, satiety andalso, human health. Using comfort foods as examples, in the first part of the lecture, methods how to unravel complex food flavors by breaking down the overall flavor sensation into single, “chemical” responses will be presented, followed by approaches how to use this knowledge in chemistry to improve the quality of the respective food. In the second part of the talk, recent results are discussed with special emphasis on compounds displaying flavor activity as well as postprandial bioactivity in the human body. The talk will, thus, include data on the fate of aroma compounds in the human body as well as on “flavor perception” in other human tissues.

L-38
STRATEGIES FOR FLAVOUR AND OFF-FLAVOUR DETECTION

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Strategies for Flavor and Off-Flavor Detection Odours and odour impressions both in a positive and in a negative way have drawn the attention of mankind since ancient times. Not only the positive changes of food smell by the cooking process but also the smell of certain plants and oils have played an important role for centuries. The odour active substances belong to the volatile fraction of a food product. This is normally the smallest part of the product (usually in the milligram per kilogram range, except in spices where the volatile fraction can be up to five percent of the whole product), nevertheless this is the driving part which is responsible for the acceptance or rejection by consumers. Off-flavours and taints are defined as unpleasant odours or tastes, the first resulting from the natural deterioration of a food, the second from its contamination by some other chemical. Although the mass range of odour active substances is limited to 300 Dalton there are literally hundred or thousand compounds having different structure, polarities and hetero atoms, so the chances of spectroscopic and chromatographic interferences is quite likely. Another restriction of analytical approaches is the fact that the method must reach the limits of detection which should be in the range of the sensory threshold. This range spans several orders of magnitude from nanogram per kilogram (or even below) to the upper milligram per kilogram range. In this presentation strategies for the determination of odours and off-odours will be discussed. Presenting several examples for pleasant and unpleasant smell in food products should demonstrate the problems and the approaches to get reliable results. Beside the sample preparation steps the focus on this presentation will be given on the separation and the detection of the target substances.

Keywords: *Odour, off-odour, GC–MS, multidimensional GC, sensory threshold*

L-39*
THE AROMA VALUE OF AROMA-ACTIVE ESTERS – AN APPROPRIATE MEANS TO ASSESS THE AROMA QUALITY OF APPLE JUICES FROM CONCENTRATE

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An inappropriate re-aromatization of apple juices from concentrate may affect the aroma quality. To elucidate changes caused by the processing steps and to define parameters for re-aromatization, the odor-active compounds in apple juices from the same batch of apples, but processed in different ways, were investigated. Because esters are known as important aroma compounds in apple juice a fast, multicomponent stable isotope dilution assay, based on headspace-solid phase micro extraction (HS–SPME) in combination with comprehensive two-dimensional gas chromatography-time of flight-mass spectrometry (GC×GC–TOF–MS) was developed. The new method enabled a high throughput of samples due to the absence of any sample work-up. So far eight new isotopically labeled internal standards were synthesized to achieve the labeling needed for electron impact ionization mode by a TOF–MS. Results on a correlation between the overall aroma of the apple juices and the quantitative data on 15 esters in apple juices will be reported. Furthermore, additive effects of esters on the overall aroma quality were investigated in model experiments ruling out some esters as markers for the juice quality.

Keywords: *Apple juice, ester, quality, TOF*

L-40
PROFILING ANALYSIS OF PINEAPPLE (*ANANAS COMOSUS* (L.) *MERR.*) FRUIT VOLATILES ALONG THE SUPPLY CHAIN USING SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY (HS–SPME–GC/MS)

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Pineapples are valuable tropical fruits appreciated by many consumers, also due to their distinct aroma quality. However, fully ripe fruits exhibiting the most intense flavour impression are only available as a premium niche product in the high-end segment, as they are to be exported by rapid cargo-planes. In contrast, the major part of fresh pineapple fruits are harvested at an earlier green-ripe stage and exported by cost-efficient reefers (in cooling containers). Consequently, for flavour development, green-ripe fruits need to be post-harvest ripened; however, may not reach the same sensory quality as fully ripe pineapples. In the present study, volatile profiles of three post-harvest stages of green ripe MD2 ("Extra-Sweet") pineapples were compared to fully ripe air-freighted fruits. Using HS–SPME–GC/MS, 142 volatiles were detected, and subsequently subjected to partial least squares discriminant analysis (PLS–DA) and partial least squares regression (PLS) in order to assess the effects of on-plant maturation and post-harvest storage on pineapple volatiles. Tentative marker compounds to discriminate the ripening stages were identified according to Vervoort et al. [1]. Total amounts of δ-octalactone, γ-nonalactone, and an isomer of 1,3,5,8-undecatetraene detected in air-freighted pineapples clearly exceeded that of all green-ripe fruits. Two other lactones, namely γ-decalactone and γ-dodecalactone, were exclusively found in fully ripe pineapples. These volatiles exhibit low odour detection thresholds and pleasant odour qualities, and thus have been described as potent contributors to pineapple flavour [2,3]. Furthermore, volatile profiles of fully ripe fruits were characterised by considerable amounts of methyl 3-(methylthio) propanoate and medium chain aliphatic methyl esters (C8, C9, C10). In contrast, alcohols, acetates, hydroxylated, acetoxylated and methyl-branched compounds such as e.g. diastereoisomeric methyl 3-acetoxy-2-methylbutanoates were predominantly present in post-harvest stages of green-ripe fruits. Based on the peroxisomal degradation pathway of methyl-branched amino acids described by Gerbling & Gerhardt [4], formatio n of selected volatiles during post-harvest storage is suggested. Application of multivariate statistical methods on HS–SPME–GC/MS profile data allowed the unambiguous distinction of all post-harvest stages of sea-freighted pineapples from fruits harvested at full maturity. Based on these findings, air-freighted pineapples can be clearly differentiated from fruits exported by reefers.

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Keywords: *Pineapple Flavour Biogenesis, Ripening Influence, Flavour Analysis, Multivariate Statistical Analysis, Gas Chromatography–Mass Spectrometry*

L-41
ANALYSIS OF VOLATILE LIPID OXIDATION PRODUCTS. IS STATIC HEADSPACE STILL FIT FOR THE PURPOSE?

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Volatile lipid oxidation products (VLOP) are important compounds from the point of view of consumers, as well as producers of various foods of various fat contents. Volatile lipid oxidation products are responsible for the characteristic odor of rancid food and are an indicator of oxidative changes in food lipids. They comprise of mainly alkanas, alkenals, alkadienals, ketones and alcohols of different sensory impact. Hexanal is a compound that is often used as an indicator in monitoring oxidation in foods, however also ratio of selected aldehydes, or profile of VLOPs is determined to monitor these processes. Static headspace was the first headspace method used for analysis of volatile lipid oxidation products, and still is used for this purpose, although the main flaw of the method is relatively low sensitivity, which is highly dependent on the food product (matrix). To overcome this disadvantage static headspace combined with analytes preconcentration on various traps have been introduced. Static headspace autosampler equipped with Tenax trap option (trap is used for multiple adsorption of volatiles from the headspace loop) coupled to GC/MS was used for experiments. Analyses were performed using a mixture of 20 volatile lipid oxidation products spiked into different matrices. Different food matrices (water, rapeseed oil, potato chips and mayonnaise) were compared in terms of extraction efficiency. It was dependent on fat contents in mayonnaise, chips/water proportion in prepared sample and was highly dependent on the analyzed compound. Standard static headspace method was optimized for analysis of tested VLOP in oil (40 min., 90°C) and compared to method that utilizes multiple adsorption of volatiles into a Tenax trap and their subsequent desorption. Optimal number of trappings (7), desorption temperature (275°C) and other parameters, such as carry over, column overloading, were evaluated. For almost all compounds "classical" headspace approach (with loop injection) provided excellent linearity in a range 0.1–50 mg/L and sufficient sensitivity (LOQ 0.05–0.3mg/L, depending on a compound). However, trapping of volatiles resulted in 10–33 increase in sensitivity of the static headspace method and allowed quantitation of compounds hard to analyze by static headspace (2,4-decadienal). The results obtained by both static headspace approaches and compared and discussed with SPME used for the analysis of the same compounds Combining static headspace analysis with multiple adsorption on adsorbents such as Tenax in a single autosampler is a valuable development in static headspace technique providing significant increase in sensitivity for volatile lipid oxidation products in food.

Keywords: *Static headspace, lipid oxidation, SPME*

L-42
POTENTIAL AND ADVANTAGES OF USING A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PRE-SEPARATION STEP, PRIOR TO COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY–MASS SPECTROMETRY ESSENTIAL OIL ANALYSIS

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The present work is focused on the off-line combination of high performance liquid chromatography (HPLC) and comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry (GC×GC-quadMS), and its application to the detailed qualitative analysis of two genuine *Citrus* essential oils, bergamot and sweet orange. Specifically, a silica column was exploited for the separation of the essential oil constituents in two groups, namely hydrocarbon and oxygenated compounds. After, each HPLC-fraction was reduced in volume, and then subjected to cryogenically-modulated GC×GC-quadMS analysis. The volatiles were separated on a normal-phase GC×GC column train set, and identified through database matching and linear retention index information. The concentrated HPLC fractions gave origin to unexpectably-crowded chromatograms, due to two fundamental GC×GC characteristics, namely the enhanced separation power and sensitivity. The results attained were particularly stimulating with regards to the oxygenated compounds, namely those constituents which contribute most to the essential oil aroma, and are of more use for the evaluation of quality and genuineness.

Keywords: *Comprehensive two-dimensional gas chromatography; baby food; quadrupole mass spectrometry; Citrus essential oil.*

Acknowledgement: *The Project was funded by the Italian Ministry for the University and Research (MIUR) with a FIRB "Futuro in Ricerca" Project n. RBFR10GSJK "Tecniche Analitiche Avanzate per l'Analisi dei Contaminanti negli Alimenti".*

L-43
MYCOTOXINS AND THEIR METABOLITES: FROM TARGETED TO UNTARGETED ANALYSIS

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Several highly publicized worldwide incidents related to chemical contaminants such as mycotoxins in food and feed have attracted much media attention. Mycotoxins are natural secondary metabolites produced by fungi on agricultural commodities in the field and during storage under a wide range of climatic conditions.
Until recently, most of the available analytical methods (e.g. HPLC–UV/FLD) for the determination of these toxic metabolites only covered single mycotoxin classes (e.g. aflatoxins, type-B trichothecenes or fumonisins). In the meanwhile, mass spectrometry based analytical methods (GC–MS, Q–TOF, LC–MS/MS) have been key for the determination of a variety of mycotoxins and their metabolites in plants and foods and for the investigation of the metabolism of these toxic compounds in body fluids such as serum and urine. One example is a multi-analyte LC–MS/MS method which has recently been developed by us and which is capable of determining 360 fungal, bacterial and plant metabolites, respectively, in cultures, cereals and food products. LC–MS- based screening has also been playing a vital role in the discovery of novel mycotoxin conjugates so called “masked” – forms of mycotoxins in the past and it is believed that this will also continue in the future.
Metabolomics has emerged as the latest of the so-called –omics disciplines and shows great potential to determine hundreds to thousands of metabolites at once over a wide range of concentrations. In this context, *in-vivo* stable isotopic labelling in combination with LC–HRMS turned out to be a powerful tool for the untargeted screening of biotransformation products of natural toxins. After measurement of biological/food samples treated with a 1+1 mixture of labelled and non-labelled precursors, labelling-specific isotopic patterns can be reliably and automatically detected by means of the novel software tool (“MetExtract”), which was developed by us. In a preliminary study, the great potential of the presented approach is further underlined by the successful and automated detection of eight novel plant-derived biotransformation products of the most prevalent *Fusarium* mycotoxin deoxynivalenol (DON). The detection of the DON-GSH conjugate and derived processing products in wheat has been reported for the first time, providing evidence for glutathione-mediated metabolism of DON *in planta*. The relevance of these novel metabolites for food safety is still to be investigated.

Keywords: *LC–MS/MS, LR-HR-MS, DON-GSH, masked mycotoxins*

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L-44
ENNIATINS IN GRAIN – EMERGING TOXINS OR JUST PEPTIDES FROM FUNGI? WHAT CAN (BIO-)CHEMICAL ANALYSIS TELL US?

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The enniatins are cyclic depsipeptides produced by different fungi with a wide range of interesting biological activities. Enniatins have been classified as “emerging toxins” after it had been shown that they occur in considerable amounts in field grain as a result of infection with certain Fusarium fungi. Contamination with high levels of enniatins (i.e. mg/kg concentrations) is rather common in grain in Northern Europe, but has also been reported in different commodities from Spain and Northern Africa. However, the widespread occurrence of the enniatins does apparently not result in acute intoxications. During the last ten years we have therefore studied the biochemical and pharmacological properties of these compounds in order to determine if they may be of any concern for consumers due to chronic exposure. The hepatic biotransformation of enniatins as well as the key enzymes associated with phase I metabolism reactions were characterised in rat, dog, human, and chicken liver microsomes. Cytochrome P450 reaction phenotyping using chemical inhibitors selective for human enzymes highlighted the importance of CYP3A4, CYP1A2, and CYP2C19 for enniatin B biotransformation. Additionally, species differences were observed both in the in vitro metabolite profiles and kinetic parameters indicating significant differences also in in vivo metabolism of the enniatins. In total 13 metabolites have been tentatively identified using liquid-chromatography coupled to ion-trap or high-resolution mass spectrometry. The microsome model used in these studies successfully predicted the metabolite profile determined in in vivo samples from a feeding study in broilers and laying hens. Intestinal absorption characteristics of enniatins were investigated in a Caco-2 transwell model. The oral bioavailability of enniatins can be influenced by cellular efflux. Thus, the active carrier-mediated efflux of enniatins across the Caco-2 monolayer was evaluated by measuring the apical-to-basolateral transport ratio in the absence/presence of known inhibitors of efflux pumps. In addition, genetically engineered Madin Darby canine kidney cells (MDCKII) expressing relevant human ABC transporters were used to confirm the ability of P-glycoprotein (Pgp), multidrug resistance associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) to mediate the secretion of enniatin B1 in vitro. Exposure of the murine monocyte-macrophage cell line RAW 264.7 with enniatin B showed that the compound may induce cell cycle arrest, cell death, and inflammation. Mechanistic studies using Caco-2 cells identified lysosomal destabilisation as a key element in enniatin B induced cytotoxicity, starting a non-apoptotic cell death pathway with morphological features previously considered as necrotic (“programmed necrosis”). With this presentation we would like to summarise our research on enniatins from the last decade.

Keywords: *Enniatins, Intestinal Apsorption, Metabolism, Cytochrome P450, Bioassay*

L-45*
MULTI-ANALYTE U-HPLC–HR/AM–MS/MS METHOD FOR CONTROL OF PESTICIDE RESIDUES, MYCOTOXINS AND PYRROLIZIDINE ALKALOIDS IN PHYTOPHARMACS/HERBAL FOOD SUPPLEMENTS

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Herbal food supplements and phytopharmaceuticals have become an important part of consumers' health care in recent years and their popularity is increasing as well as the size of the market. However, under certain conditions positive effects of biologically active components contained can be overshadowed by the presence of various contaminants which can pose a health risk for consumers. Besides of heavy metals and pesticide residues, also occurrence of ´natural´ contaminants such as mycotoxins and/or some toxic plant species have been found in various commercial products. Worth to notice, that contrary to staple food, systematic chemical safety control of food supplements/phytopharmaceuticals is practically lacking. Worth to notice that food analysis of such difficult, largely variable matrix is a challenging task. Although in most existing studies, multi-analyte methods were employed, typically only one group of the above hazardous compounds was targeted. In this study, we merged target analysis of 323 pesticide residues, 56 mycotoxins and 11 pyrrolizidine alkaloids into a single method. For isolation of these analytes, QuEChERS–like extraction method was used, obtained extract was then examined by ultra-performance liquid chromatography coupled with tandem high resolution mass spectrometric detection (U-HPLC–HRMS/MS) employing quadrupole-orbitrap hybrid mass spectrometer Q-Exactive™. The performance characteristics of the implemented procedure are thoroughly discussed and critically compared with those, achievable by alternative determinative MS-based approaches. The overview of the results obtained by screening of most popular herbal food supplements available at the Czech market, such as those based on Echinacea purpurea, Gingko biloba, etc is provided.

Keywords: *Pesticides, mycotoxins, herbal food supplements, phytopharmaceuticals, U-HPLC–HRMS/MS.*

Acknowledgement: *Financial support from specific university research (MSMT No 20/2013).*

L-46
NEW INSIGHTS INTO THE FORMATION OF FB1 – FATTY ESTER DERIVATIVES AND THEIR RELEVANCE IN MAIZE

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Fusarium verticillioides, together with *F. proliferatum*, are filamentous fungi able to colonize maize crops with the simultaneous fumonisin accumulation in kernels. Since 1988, over 28 fumonisin analogues were identified and classified into four groups, named A-, B-, C- and P-series. The great attention received by B-series fumonisins is due to their ascertained toxicity on humans and animals. Concerning minor fumonisin analogues, since they are commonly produced at relatively low levels, they have been less extensively studied than B-series analogues so far. Recently, the occurrence of three fumonisins derivatives obtained by the esterification of FB1 with palmitic, oleic and linoleic fatty acids were detected and characterized in solid cultures of F. verticillioides grown on cracked rice. In this study, the natural occurrence of such derivatives, namely palmitoyl-, oleoyl- and linoleoyl-EFB1, in raw maize will be described. In addition, information about the role played by the plant-pathogen cross-talk in their formation will be given. Finally, since these compounds could be regarded as masked mycotoxins, their relevance for food and feed will be critically discussed.

Keywords: *Masked mycotoxins, corn, plant-pathogen interaction, Fusarium*

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L-47*
IMPROVING ON-SITE MYCOTOXIN MEASUREMENTS BY COMBINING RAPID ANALYSES WITH DUST SAMPLING

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On-site, mycotoxin measurements shall enable rapid decisions e.g. on the acceptance or rejection of lots. Hence, results have to be fast available, easy to get and, first of all, reliable. The reliability of measured mycotoxin contents depends on the uncertainties arising from all parts of the measurement process. Whereas the quality of rapid tests for on-site analyses has steadily improved within the last years, sampling remains the most critical step in the whole analytical chain. Especially for heterogeneously distributed storage mycotoxins, common sampling procedures are either not representative or, like the commission regulation (EC) 401/2006, ensure representativeness but are hardly applicable in terms of needed workforce and time. An innovative approach using dust samples was developed and tested for mycotoxin analyses of food and feed bulk-ware. Small particles are abraded from the surface of e.g. grain or raw coffee beans during every transport and handling step. Hence, these dusts arise from a huge number of grains and can represent the whole lot better than a limited number of grain samples. A strong enhancement of mycotoxin contents on the small, surface particles compared to the bulk material was observed. Furthermore, dusts generally contain minor levels of starch, proteins, and fats. Hence, the natural accumulation of mycotoxin in the dust and its limited matrix effects facilitate analyses of mycotoxins with rapid test systems even at low legal limits. Data models were developed by analysing corresponding dust and bulk-ware samples for mycotoxins by means of HPLC–FD or HPLC–MS/MS multi-toxin screening methods. As the contamination of the overall sample and its dust particles correlated, contaminations in the bulk were calculable from concentrations determined in respective dust particles. Data models were set up for wheat, maize, and green coffee beans for main fusarium toxins, aflatoxins and/or ochratoxin A. On-site, grain- and dust samples were taken during the unloading of trucks. A dust-sampler was customised to separate dust with specific particle sizes to avoid cross-contamination via fine dusts. In contrast to grain samples, no additional physical sample preparation or homogenization step was needed for the dust. Instead, the sample was directly extracted and analysed using common lateral flow test systems. The results were recalculated using the data models and compared to results obtained from the grain samples. In this way, the applicability of the new approach combining rapid analyses with dust sampling was shown. The innovative high-throughput technology has the potential to improve on-site mycotoxin measurements in terms of speed, sensitivity, manageability and reliability and thus is a promising tool for enhanced industrial self-control.

Keywords: *Mycotoxins, dust, sampling, representativeness, rapid tests*

L-48
DISCOVERY AND TOXICITY ASSESSMENT OF NOVEL MYCOTOXINS PRODUCED BY US ISOLATES OF FUSARIUM GRAMINEARUM

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Fusarium graminearum is a filamentous fungus, wide-spread across temperate regions of the world, and can produce several mycotoxins on almost every cereal. Most importantly, the trichothecenes deoxynivalenol (DON) and its biosynthetic precursors 3-acetyl-DON and 15-acetyl-DON act as virulence factors for Fusarium Head Blight Disease of wheat. The toxins, when taken up in food or feed, strongly inhibit eukaryotic protein synthesis and cause vomiting as well as anorexic and immunosuppressive effects in humans and animals. From 2003 to 2006 a large scale survey of *F. graminearum* from fields in the northern US was conducted. In some strains which were genotyped to be 3-acetyl-DON producers no known trichothecene could be detected. Still, the strains showed normal disease symptoms on wheat, suggesting that a novel trichothecene mycotoxin might be formed by them. LC–MS measurements revealed novel compounds in extracts of rice cultures, which were inoculated with the newly isolated strains compared to a reference *F. graminearum strain*. Using LC–HR–MS/MS a typical fragmentation pattern of trichothecenes was obtained for one compound, termed NX-2, and its sum formula was elucidated as $C_{17}H_{24}O_6$. Database search indicated the same sum formula for the known mycotoxins 4- and 15-monoacetoxy-scirpenol, which were ruled out by the measurement of standards. After optimizing production and extraction conditions, NX-2 was purified from inoculated rice cultures by normal phase and subsequent reversed phase chromatography. Its chemical structure was elucidated by 1D- and 2D-NMR measurements. NX-2 has the characteristic backbone structure of a 12,13-epoxytrichothec-9-ene, an acetyl-group at C-3 and hydroxyl-groups at C-7 and C-15. Compared to 3-acetyl-DON it lacks the keto group characteristic for type B trichothecenes. As it is expected that NX-2 is rapidly deacetylated in planta, its deacetylated form (dNX-2) was produced by alkaline hydrolysis. The toxicities of the novel mycotoxins were evaluated using an in vitro translation assay. dNX-2 had an approximately two-fold decreased potency to inhibit protein biosynthesis than DON. Inoculation of wheat ears with the isolated strains revealed the presence of dNX-2 as main compound up to levels of 500 mg/kg. The natural occurrence of NX-2 and dNX-2 is currently evaluated.

Keywords: *Mycotoxins, Fusarium, toxicity, novel compound, structure elucidation*

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L-49
INDIRECT METHODS FOR THE DETERMINATION OF CONJUGATED FORMS OF DEOXYNIVALENOL IN CEREALS

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Mycotoxins, the toxic secondary metabolites of filamentous fungi, contaminate a wide range of food and feed commodities. These natural toxins may occur not only in native forms, but also as so-called masked mycotoxins. Masked mycotoxins are formed in planta by linking small polar compounds to the mycotoxin molecule as a protective response. The chemical modification leads to decreased toxicity for plants. Masked mycotoxins are than stored in vacuoles (extractable conjugated forms) or covalently or non-covalently bound to macromolecules (non-extractable bound forms). Extractable conjugated forms can be detected by appropriate analytical methods when their structure is known and analytical standards are available. Non-extractable bound mycotoxins cannot be extracted directly and have to be released from the matrix by chemical or enzymatic treatment prior to chemical analysis. The best known masked mycotoxin, deoxynivalenol-3-β-D-glucoside (D3G), is formed in DON-contaminated wheat grains. Moreover, increase of D3G levels during malting and brewing was observed and also the presence of DON-diglucosides and DON-triglucosides in malt and beer was revealed. Recently, several other masked DONs were found to be formed in wheat (DON-S-cysteine, DON-S-cysteinyl-glycine, DON-glutathione). All those masked forms potentially might be cleaved to DON during food processing or hydrolyzed in the digestive tract of mammals and thus contribute to the total dietary DON exposure. Indirect methods aim to determine the entire pool of masked mycotoxins in a sample by their conversion into the native toxin using chemical or enzymatic hydrolysis. This contribution aims to provide a critical assessment of three indirect methods for total DON determination based on acidic hydrolysis using trichloroacetic acid, trifluoroacetic acid or trifluoromethanesulfonic acid. The stability/degradation of DON, D3G, 3-acetyl- and 15-acetyl-DON was assessed on spiked samples of wheat, maize and barley using an LC–MS/MS method which has been developed and validated for this purpose. Concerning potential enzymatic hydrolysis, the capability of several glucosidases of different classes to cleave D3G in wheat flour extract was tested. Judging from our experiments, neither acidic nor enzymatic hydrolysis methods for the quantification of the total amount of DON in cereals are recommended.

Keywords: *Masked mycotoxins, chemical hydrolysis, enzymatic hydrolysis, deoxynivalenol-3-glucoside, LC–MS/MS*

L-50
APPLICATION OF NEWLY DEVELOPED UPLC AND CHIP BASED MICROUPLC TRIPLE QUADRUPOLE MASS SPECTROMETRIC METHODS FOR THE DETECTION OF VARIOUS CLASSES OF MARINE LIPOPHILIC BIOTOXINS IN SHELLFISH AND FISH

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Marine biotoxins are produced by phytoplankton and can accumulate in shellfish or fish. The toxins accumulate in shellfish such as mussels, oysters and clams via filter feeding. Fish accumulates via herbivorous fish grazing on benthic algae which are in their turn consumed by higher trophic fish such as red snappers. If contaminated shellfish or fish is consumed severe intoxication syndromes may occur. Within Europe three intoxication syndromes related to shellfish consumption may occur, amnesic shellfish poisoning, paralytic shellfish poisoning and diarrheic shellfish poisoning (DSP). DSP toxins are belonging to the class of lipophilic marine biotoxins which represents a larger group of marine biotoxins such as azaspiracids, yessotoxins, pectenotoxins and cyclic imines. Furthermore, cigautoxin that occur mainly within fish from tropical regions also show lipophilic properties and structural similarities. Ciguatoxin is toxic at low levels and adverse effects are diverse such as gastrointestinal disorder, neurologic and cardiovascular symptoms. For most of the lipophilic marine biotoxins LC–MS/MS methods have been developed. The official EU reference method for lipophilic marine biotoxins with the exception of cyclic imines and ciguatera is LC–MS/MS based. This reference method is based on a fixed extraction procedure followed by separation using conventional LC with either an acidic mobile phase or alkaline mobile phase followed by tandem quadrupole mass spectrometric detection. The aims are to produce a much faster routine analysis than the conventional LC method using UPLC technology. The developed UPLC–MS/MS method (5 min) includes regulated as well as some non-regulated toxins that are of interest for EFSA. The non-regulated are belonging to the class of the cyclic imines. Results will be shown of the application of this improved UPLC methodology in routine applications for various incidents that occurred in the course of 2012–2013. Furthermore, the conventional LC method is also transferred to so called chip based microUPLC. The rationale behind this research originates from both a green analytical chemistry philosophy (reduced solvent use) and from the limited sample extract volumes available when expensive standards are used. Our chip based UPLC–MS/MS method reduces the solvent usage by more than 90% and also the amount of extract or standard injected is reduced from 5 µL to 0.5µL. Especially, for the analysis of ciguatoxins for which semi-purified toxin standard is extremely expensive (€ 1500/µg) this reduced sample usage is of great importance. The robustness, sensitivity and applicability of these 150 µm chip based microUPLC formats for the analysis of marine biotoxins will be presented.

Keywords: *Lipophilic marine biotoxins, ciguatoxins, chip based UPLC, tandem mass spectrometry*

L-51
A PROTEOMICS APPROACH FOR THE QUANTIFICATION OF STAPHYLOCOCCUS AUREUS ENTEROTOXINS A AND USING UPLC–MSMS ANALYSIS

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In number of foodborne outbreaks *S. aureus* intoxications stand highly ranked next to those caused by other pathogens like *Bacillus cereus, Escherichia coli, Salmonella enterica* and others. In number of outbreaks and human cases per causative agent in strong evidence food-borne outbreaks in the EU, bacterial toxins share the second place together with foodborne viruses. Among bacterial toxins, staphylococcal enterotoxins (SEs) was by far the most important etiological agent. The largest proportion of strong evidence outbreaks caused by staphylococcal toxins was attributed to mixed or buffet meals (28.9%), followed by cheese (18.4%). This indicates that milk, milk derived products and ready-to-eat (RTI) foods require attention in detecting and quantifying SEs. We developed and are validating method to specifically quantify SEs in pork meat using stable isotope dilution and UPLC–MSMS analysis. Pork meat was spiked with SEA and/or SEB. Next we extract the SEs from the meat using isoelectric point precipitation and ultrafiltration. Afterwards the sample was overnight digested with trypsin to obtain SE-derived peptides. Only the endogenous peptides that are unique to their respective SE are selected for analysis with LC–MSMS. Additionally all samples are spiked with internal standards (IS). These IS are isotopically labelled equivalents of the unique endogenous tryptic peptides and show the same chemical behavior as the endogenous peptides. Both the endogenous peptide and its respective IS should elute from the column at the same retention time, assuring high specificity of the method. The sample is then injected and analysed using online Solid Phase Extraction (SPE) –UPLC–MS/MS. Analytes of interest are trapped on the SPE-column, while e.g. interfering salts are flushed to the waste. Afterwards, the peptides are back-flushed from the SPE-column onto the analytical column where the separation occurs. The MS/MS is programmed to search for the parent-daughter mass transition for each unique endogenous peptide and IS. These parent-daughter transitions are unique for each peptide and can be used to detect the SEs very specifically. We are currently validating this method according to ISO 2002/657/EC. When comparing the MS spectra peaks found for the IS and the endogenous peptides we were able to identify and quantify the presence of SEA and/or SEB in the spiked meat. We are in the process of method validation where we will determine linearity, matrix effect, repeatability, trueness, recovery, LOD and LOQ of the method. We developed and are validating method to specifically detect SEs in pork meat. This method could be used to quantify SEs in food poisoning once the presence of *S. aureus* is proven or it could also be used (in parallel with current immunologic methods) to directly confirm and quantify SEs. Currently, only SEA and SEB are identified and quantified, but the method will be expanded to other SEs for which antibodies do not yet exist.

Keywords: *Staphylococcal enterotoxins, LC–MSMS, isotope dilution, proteotypic peptides*

L-52
EVALUATION AND IMPLEMENTATION OF THE SAMPLE PREPARATION STEPS FOR THE LC–MS/MS ANALYSIS OF CIGUATOXINS PRESENT IN FISH SAMPLES

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Ciguatera fish poisoning (CFP) is associated with an intoxication caused after the consumption of certain fish species inhabiting tropical and subtropical regions contaminated with ciguatoxins (CTXs). These toxins are produced by species of dinoflagellates Gambierdiscus toxicus, which are accumulated in the fish tissue. Ciguatoxins already represent a concern in several geographic areas but these toxins have recently emerged in the EU and their presence is being presently investigated in several coastal areas, in particular in Canary Islands (Spain). LC–MS/MS has been proposed as analytical tool very valuable for the analysis of these toxins. The lack of standards and reference materials is the main limitation encountered at present for the progress in the implementation of this methodology, nevertheless big progress have been made in the implementation of LC–MS/MS conditions based of the limited standards already available from particular sources. Evaluat ion and Implementation of sample pretreatment protocols have been identified as a critical need for the advances in the analytical development, thus this work is focused on evaluation of different extraction and cleanup procedures already described in the literature with the aim of proposing the approach that would contribute more efficiently to the LC–MS/MS results. The results presented in this study show the influence of the extraction and clean up conditions in the recovery yield, the optimization of these conditions clearly contribute to an increased sensitivity of the LC–MS/MS analysis. Despite of the limitation of the lack of standards for all the ciguatoxins potentially involved in such contaminations, improved sample preparation protocols will allow to discriminate among some of the ciguatoxins present in these complex matrices, thus representing an important contribution and advance in the analysis of these toxic compounds which are emerging in the EU.

Keywords: *Ciguatoxins, Sample preparation, LC–MS/MS*

L-53
A NOVEL METHOD FOR THE SIMULTANEOUS DETERMINATION OF 14 SWEETENERS USING UPLC–MS/MS WITH ONLINE SPE

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Non-nutritive or low calorie sweeteners are commonly used world wide in the food industry, often in combination in order to limit undesirable tastes. The list of allowable sweeteners varies among nations worldwide and it is important for the FDA to monitor these highly consumed products, especially imports, to ensure that they are in compliance with US regulations. Current analytical methods for confirmation and quantifying sweeteners are outdated and do not provide confirmation of analyte identity, required for robust regulatory actions. There is a need for an LC–MS/MS method for the simultaneous determination and confirmation of 14 sweeteners in highly consumed foods such as diet beverages, candies and yogurts.
A novel method has been developed for the determination of aspartame, acesulfame K, cyclamate, alitame, neotame, dulcin, sucralose, saccharin, neohesperidine dihydrochalcone, rebaudioside A, stevioside, xylitol, maltitol and erythritol. A reversed-phase UPLC column has enabled the separation of target analytes using a gradient within 30 minutes, and electrospray ionization MS/MS in negative mode. Deuterium labeled saccharin, cyclamate and C13 labeled sorbitol are used as internal standards. The MS parameters have been optimized for all 14 sweetener compounds. The method has been developed using an Agilent 1290 UPLC interfaced with an AB Sciex 4000 Mass Spectrometer. The method has been applied to the analyses of drinks by dilution and filtration prior to UPLC–MS/MS analysis.
The sample preparation and cleanup of yogurt samples is performed using an automated sample preparation system manufactured by Gerstel, Inc. This automated online SPE procedure uses a C18 end-capped cartridge for sample cleanup. The linear range for the 14 target compounds spans 25 - 2000 ng/mL. Spike and recovery studies were conducted in both carbonated and non-carbonated beverages. The drinks were spiked at the maximum usable concentrations governed by the European Union (EU). In the case of the sugar alcohols that have quantum satis (harmless therefore no specific allowable limit) in the EU, the spiking concentration chosen was 20 ppm. This method is specific, reproducible (% RSD values range from 5-15) and sensitive. It is a tool for detecting adulterated or misbranded foods for both domestic and imported products. The development of modern and reliable methods for the quantitative determination of non-nutritive sweeteners in foods will allow the FDA to more efficiently monitor the food supply and make regulatory and compliance decisions.

Keywords: *Sweeteners, UPLC, Automated online SPE*

L-54
ANALYTICAL STEPS DEVELOPED FOR INVESTIGATING NEW SOURCES FOR ALGAE BASED DIETARY SUPPLEMENTS

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In the recent decade, consumers´ interest to support their health through the use dietary supplements continues to increase. In this context, searching for new sources of biologically active substances with positive health effects realizes a great challenge. Micro-algae have emerged as a significant source of highly valuable molecules, and several types of algae (*Chlorella sp., Spirulina sp., Ulkenia sp., Haematococcus sp.*, etc.) are nowadays used as sources of proteins, carbohydrates, minerals, vitamins, and/or lipids with high content of polyunsaturated fatty acids and carotenoids. Nevertheless, our preliminary studies have indicated that the potential of microalgae to be employed as an untraditional source of nutrients can be much broader: new, so far unexplored algae exist in nature as well as in microorganism collections. This presentation will demonstrate a comprehensive analytical strategy we have used in investigation of bio-matrix for which the composition is completely unknown. The following steps were tested and critically evaluated: (i) various ways of mechanical treatment of algae biomass to disintegrate the cell walls and increase the extraction efficiency, (ii) extraction-fractionation steps enabling isolation of polar, medium-polar, and non-polar compounds, and selection of the optimal approach with regards to, large-scale industrial application, (iii) non-target screening of fraction components followed by assessment of different forms of identified compounds employing ultra-performance liquid chromatography coupled with high resolution tandem mass spectrometric detection (U-HPLC–HRMS/MS) or high resolution ambient mass spectrometry (DART-MS), (iv) assessment of biological activity of extracts by a set of antioxidant activity / enzyme inhibitory tests. For development of this highly efficient screening strategy, model microalgae with high potential of eicosapentaenoic fatty acid production, specifically (*Trachydiscus minutus*), were used.

Keywords: *Biologically active compounds, biological activity, algae, extraction, dietary supplements, U-HPLC–HRMS/MS*

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L-55*
FOODOMICS TO UNRAVEL THE REGULATING ROLE OF PHYTOHORMONES IN CAROTENOIDS' METABOLISM IN TOMATO FRUIT

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Carotenoids are important secondary metabolites, which are mainly synthesized by plants, algae and certain types of bacteria and fungi. In epidemiological and clinical studies, associations were found between the intake of the concerned dietary compounds and the prevalence of chronic-degenerative diseases. Because of these beneficial health effects, carotenoids are intensively studied to better understand amongst others their partitioning and metabolism in plants. In this context, phytohormones might fulfil an important role since these compounds have a regulatory function in various plant processes. This study aimed for a better understanding of carotenoids' metabolism by investigating the regulating role of phytohormones in the occurrence of tomato fruit carotenoids. The realization of the outlined objective completely fits into the foodomics concept, which is intended to be a global discipline that includes all of the emerging working areas in which food, analytical techniques and bioinformatics are combined. Completing this aim required the development of analytical methods for quantification of both carotenoids and phytohormones, present in tomato plant tissue. For the extraction of phytohormones (with a representative for each hormonal class), the developed protocol included solid liquid extraction with Bieleski solvent (methanol/water/formic acid), a purification step using a 30 kDa Amicon centrifugal filter unit, and an enrichment phase. Extracts were analyzed using UHPLC–Orbitrap Exactive^{TM} MS. The high resolution (up to 100,000 FWHM) and the full-scan princ iple of the applied mass spectrometer were particularly suited to realize a metabolomic approach. The protocol for extraction of the carotenoids (lutein, zeaxanthin, α-carotene, β-carotene, lycopene) was developed by means of a factorial d-optimal design and consisted of liquid-liquid extraction, using methyl-tert-butyl ether and methanol. Extracts were also analyzed by Orbitrap Exactive^{TM} MS. For chromatographic separation, a C30 HPLC-column was used. Both methods were successfully validated since linearity (R^2> 0.99), repeatability (<15%), within-laboratory reproducibility (< 20%), specificity and recovery (> 85%) were satisfactory. Additionally, the obtained LOD (S/N ≥3) and LOQ (S/N≥10) values were good for all targeted analytes. Subsequently, an experiment was set up in which tomato plants were subjected to either a control or drought treatment. The purpose was to trigger a stress response in the tomato plant, resulting in altered phytohormone and increased carotenoid concentration levels. Statistical interpretation of the analytical concentrations, obtained from these fruits, allows to investigate the relation between the concerned components. To this end, suited software programs, including Sieve^{TM} (fingerprinting) and Simca^{TM} (data analysis), have been applied. The different aspects of the described foodomic approach will be discussed.

Keywords: *Tomato Fruit, Carotenoids, Phytohormones, Orbitrap Mass Spectrometry, Metabolomics*
Acknowledgement: *Institute for the Promotion and Innovation through Science and Technology in Flanders (IWT Vlaanderen), Research Foundation of Flanders (FWO)*

L-56
VALIDATION OF OMICS-BASED SCREENING STRATEGIES FOR DETECTING GROWTH PROMOTING PRACTICES IN BREEDING ANIMALS

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Although prohibited for more than 20 years within the EU, growth promoting practices for livestock fattening purposes are still suspected. Methods based on gas- or liquid chromatography coupled to (tandem) mass spectrometry are today considered as state-of the-art for monitoring, in a targeted approach, residues of known drugs. To overcome the detection of anabolic practices, new synthetic xenobiotic growth promoters have been designed and new ways of applications, such as the administration of low dose cocktails, have been developed. In this context, innovative screening strategies are urgently needed to enable efficient control of such practices. Omics have recently shown their relevance in highlighting physiological responses resulting from anabolic compounds administration, and in particular, metabolomics and steroidomics studies have demonstrated the efficiency of mass spectrometric-based profiling to discriminate anabolised from control animals [1–6]. These studies were designed to focus on the main suspected anabolic practices and to fit with potential practices in terms of compounds, doses and treatment lengths, so that steroids-based treatment have been considered, as have b-agonists or recombinant somatotropin ones. Various descriptive and predictive models have been set up, allowing efficient discrimination of the treated and control populations considered. The next challenge is now to free the different established statistical models from their respective given experimental conditions to elaborate models able to predict samples arising from other experimental conditions, i.e., other animals (age, sex, breeding conditions...), other anabolic compounds, doses, treatment lengths... Overcoming this challenge is a necessary step in the validation process of these metabolomics and steroidomics strategies before considering any official implementation of the tool for screening purposes. The objective of the presentation is to illustrate, through various examples (steroids, b-agonists, somatotropin), some validation strategies to assess biomarkers relevance and robustness upon variability induced by different animals, breeding conditions, drugs, cocktails and doses. The implementation of suspicious thresholds is described and performances of established models are discussed with regards to EU requirements for screening methods.

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Keywords: *Metabolomics, steroidomics, biomarkers, validation, screening*

L-57*
METABOLOMIC PROFILING OF THE GLUCOCORTICOID STATUS OF HOLSTEIN-FRIESIAN COWS BY HIGH-RESOLUTION MASS SPECTROMETRY UPON ADMINISTRATION OF PREDNISOLONE

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The anti-inflammatory properties of the natural glucocorticoid cortisol have led to the development of synthetic analogues, which exert even higher anti-inflammatory activities. Moreover, these drugs also induce body weight gain in production animals by improving feed intake and lowering feed conversion. Due to their growth-promoting effects, the use of synthetic glucocorticoids is strictly regulated in the European Union (CD 2003/74/EC). In the frame of the national control plans, which should ensure the absence of residues in food products of animal origin, in recent years, a higher frequency of prednisolone positive bovine urines has been observed. In an attempt to understand the origin of this prednisolone, an in-vivo study was conducted on adult Holstein-Friesian cows for further deepening the knowledge of the metabolism and distribution of prednisolone in cattle intended for meat production and to allow the characterisation of metabolites that may be u sed as a biomarker for exogenous administration. Because of the complex nature of feces and urine as biological matrices, appropriate sample preparation procedures were required, but in terms of the metabolomic approach to be kept as generic as possible. To this extent, Plackett-Burman designs were successfully applied to develop two different sample preparations protocols. Metabolomic profiling was performed by using two different high resolution mass spectrometers: a stand-alone Orbitrap (Exactive^{TM}) and a ToF–MS/MS (TripleToF^{TM}). Targeted analysis of the known glucocorticoids was successfully validated according to CD 2002/657/EC. Decision limits and detection capabilities for prednisolone, prednisone and methylprednisolone in urine ranged from 0.1 to 0.5 µg L^{-1} and from 0.3–0.8 µg L^{-1}, respectively. For the natural glucocorticoids limits of detection and limits of quantification for dihydrocortisone, cortisol and cortisone ranged, respectively, from 0.1 to 0.2 µg L^{-1} and from 0.3 to 0.8 µg L^{-1}. In feces similar resu lts were obtained. The applicability of the analytical methods for untargeted metabolomic profiling was demonstrated by using ToxID^{TM}, Sieve^{TM} (Thermo Fisher Scientific), Simca^{TM} (Umetrics) and MetabolitePilot^{TM} (AB Sciex) software, enabling an efficient screening of the full scan data. A first screening was conducted on urine and feces samples collected from 2 cows and 2 calves after oral administration of prednisolone (1 mg kg^{-1} BW). Several prednisolone metabolites were identified, including 20β-dihydroprednisolone and 20α-dihydroprednisolone. The potential of these metabolites as a biomarker for illegal administration as opposed to endogenous formation will be further confirmed in a larger in vivo design.

Keywords: *Biomarker, Metabolomics, High-Resolution Mass Spectrometry*

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L-58*
MEASUREMENT OF ALKYLRESORCINOL BIOMARKERS OF THE WHOLE GRAIN FOOD AND RYE INTAKE IN URINE BY ELISA, GC–MS AND LC–MS/MS

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Alkylresorcinols (ARs) are amphiphilic phenolic lipids that may be used as biomarkers for the intake of wholegrain wheat and rye. The parent ARs are extensive metabolized in liver and formed metabolites can be detected in plasma and urine, either as carboxylic acids or as glucuronide and sulphate conjugates. Two metabolites of interest, 3-(3, 5-dihydroxyphenyl)-propanoic acid (DHPPA) and 3, 5-dihydroxybenzoic acid (DBHA), are considered to be candidate urinary biomarkers. AR metabolites levels are currently measured by chromatographic techniques but for samples from large epidemiological studies, immunoassay techniques can be preferred. Polyclonal antibodies against the above metabolites were produced and characterized in terms of cross-reactivity, assay sensitivity, precision and accuracy. Subsequently, the developed ELISA method was used for analysis of urine samples from intervention and/or epidemiological studies being previously analysed by GC–MS. However, the ELISA showed systematically higher values compared to GC-MS results. Therefore, LC–MS/MS method using Q Exactive spectrometer was developed and innovative MASS FRONTIER v.7.0 software was used for evaluation of spectral data .The analysis of the mass spectrum provided data for tentatively identified compounds to explain several-fold-higher concentration of the metabolites measured by ELISA in comparison to GC-MS and LC–MS/MS. The so far unknown AR-related structures, derived from MS spectra, will be suggested as a potentially cross-reactants for DHPPA and DHBA in ELISA.

Keywords: *ELISA, LC–MS/MS, GC–MS, alkylresorcinol, biomarker*

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L-59
NEW LC–MS APPROACHES TO OVERCOME ANALYTICAL DIFFICULTIES DERIVING FROM THE APPLICATION OF EU PESTICIDE REGULATIONS FOR FRUITS AND VEGETABLES

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The effectiveness of the EU enforcement and risk assessment application under Regulations (EC) No: 396/2005, 882/2004, 7882/2012 etc. rely to a large degree on the adequate performance of the food control laboratories. A number of conflict issues between Regulations and their application in routine laboratories have been highlighted based in our experience as European Reference Laboratory. Some of these have been satisfactorily solved but others remained as being important analytical challenges. This presentation is focused in those analytical issues that remain as important laboratory challenges and the importance in using new mass spectrometry instrumentation to overcoming them. The utmost of these conflicts have been selected:

• Difficulties arising from complex or "difficult" matrices that typically cause serious problems in qualitative and quantitative analysis.

• Limitations in the analytical scope. Commonly as a consequence of workflow limitations to introduce a large number of compounds full validated in multiresidue methods.

• Other relevant issues commented will be difficulties deriving from the application of complex residue definitions, degradation processes etc. Selected and illustrative practical cases learnt from the 15 European Proficiency Tests performed during the last years are presented. From them a number of examples of the capabilities of update LC–MS approaches to solve the presented difficulties are also evaluated.

Keywords: *Pesticide, Regulations, LC–MS, Proficiency Tests*

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L-60*
AUTOMATED HILIC SAMPLE PREPARATION FOR PESTICIDES – DETERMINATION OF 300 COMPOUNDS FROM DIFFERENT FRUITS AND VEGETABLES WITH 2D–LC–MS/MS

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We present a fully automated system for the determination of up to 300 different class pesticides from various fruits and vegetables without any sample preparation [1]. For the measurement we inject an aliquot of an acetonitrile raw extract into the system. The following clean-up is carried out by a multidimensional liquid chromatography. The pesticides are separated from the matrix compounds by a HILIC column and transferred to the analytical RP column via a packed loop interface. Due to the chromatographic approach we obtained a significantly better cleaning effect compared to the classical QuEChERS method [2]. The matrix effect profiles of the intermediate and the final extracts were determined with the postcolumn infusion technique and compared with the QuEChERS clean-up [3,4]. With the two-dimensional approach we detected fewer matrix effects. Strong ion suppressions were detected in QuEChERS extracts even at very late retention times. With our system these matrix effects were completely removed. The method was validated for more than 300 pesticides. The majority of the tested compounds showed a recovery between 70% to 120% and good sensitivity. The two-dimensional method was able to determine high priority pesticides analogue to the established methods with analogous sensitivity but only one injection of the acetonitrile raw extract. No further clean-up was needed. In spite of the injection of pure sample extract the method was very robust. For about one week, only very difficult matrix extracts from rocket, black tea and hop (300 samples) were injected. During this time no changes in signal intensity or retention times were observed.

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Keywords: *Pesticides, multi residue analysis, multidimensional LC, automated sample preparation, HILIC*

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L-61
PERFORMANCE OF CANDIDATE DETECTORS FOR MULTIRESIDUE ANALYSIS OF PESTICIDES IN WATER BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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The determination of pesticides in water samples is challenging and requires the development of highly resolving and sensitive multiresidue methods. Due to their enhanced peak capacity and the sensitivity of cryogenic modulators, comprehensive two-dimensional gas chromatography (GC×GC) systems are well suited for multiresidue analysis in food or environmental samples. Apart from TOF–MS which is often presented as the gold standard detector to hyphenate with GC×GC, several cheaper solutions like Flame Ionization Detector (FID), Flame Photometric Detector in phosphorus (FPD/P) or sulphur modes (FPD/S), Nitrogen Phosphorus Detector (NPD), and Electron Capture Detector (ECD) can be considered as viable alternatives for pesticide determination. The aim of the present study was to discuss the relevance and the complementarities of FPD/P, FPD/S, ECD, NPD, FID and TOF–MS for the GC×GC analysis of a mix of 60 pesticides including organophosphorus pesticides, syntheti c pyrethroids and fungicides. After optimization of the GC and GC×GC setting (including column set and modulation period), the two configurations were assayed using 11 concentrations of the pesticide mix ranging from 0.1 ng/ mL to 2 µg/mL in hexane. The relative performances of the GC×GC systems were benchmarked in terms of linearity, R^2, LOD, LOQ on a selection of compounds including phosphorus, sulfur, nitrogen and one or several halogenated constituents detectable by all the configurations and compared to GC data. The GC×GC suitability of each detector, and their potential complementary attributes, is discussed based on the qualitative comparison of the contour plots of the full mix, and examination of the GC signal in terms of peak width and peak symmetry. The controversial issue of sensitivity enhancement in GC×GC was considered for optimised GC and GC×GC operation.

Keywords: *Pesticides, GC×GC, FPD P/S, NPD, µECD, TOF/MS, FID*

L-62
REVEALING THE IDENTIFICATION POWER OF COLLISION CROSS SECTION: A NOVEL APPROACH APPLIED TO PESTICIDE ANALYSIS IN FOOD

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Performing pesticide analysis in food requires coping with multi-class compounds, different matrices and responding rapidly. Screening methods are very useful as they can discriminate samples without any pesticides from those with detectable residues. The laboratory can then focus their efforts on quantitative methods for a smaller number of samples. Different strategies can be applied for screening purposes. Full scan acquisition has however driven most of the attention because of its inherent benefit of theoretical detection of unlimited number of compounds. In spite of this analytical potential, it is well characterized that many factors can influence mass spectra for LC-based methods and given the complexity of the samples analysed, reliable identification can be unreachable in some cases. Ion mobility is known to be a powerful analytical tool for the separation of complex samples and collision cross sections of compounds derived from drift time has been extensively used for characterization purposes. We will present a novel way to use these special mobility features in screening methods from acquisition to data processing. For the assay, UPLC–HDMSE experiments were performed on a Synapt G2-S using a series of standard solutions, spiked matrices and a previous proficiency test. CCS values were generated from the standard solutions and inserted into a scientific library within a new scientific information system. Then, the screening method performances were tested with samples (blank matrices, spiked samples and proficiency test). Based on these results, we will show how we can reliably reduce the number of false positive and more importantly avoid false negative identifications.

Keywords: *Multidimensional analysis, pesticide analysis, Liquid chromatography-Ion mobility-High Resolution Mass spectrometry, identity confirmation*

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L-63*
MARINE AND CYANOBACTERIAL TOXIN ANALYSIS: A NOVEL BIOANALYTICAL SUPER MOUSE

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The state-of-the-art in natural marine biotoxin analysis in seafood is now quite diverse with progress in moving away from the antiquated mouse bioassays. The future for bioanalytical methods requires cost-effective novel approaches to compete with the highly skilled multi-analyte detection now offered by laboratory based analytical approaches such as mass spectrometry. However, to realize demands for improved food security there is an increasing requirement for rapid portable tests for remote on site end product testing for managerial decisions from environment to farm to fork. To advance even further the state of the art it is no longer sufficient that rapid tests detect only one toxin or the structurally similar group. Advancements are required whereby biosensors should detect diverse groups of phycotoxins, mycotoxins or plant alkaloids as a single test. The difficulties of this approach with microarray platforms arise with regulatory limits and assay des ign. For marine and cyanobacterial toxins antibody based novel sensor methods offer model solutions but the greatest difficulty still arises with the detection of all analogues within a toxic group to meet regulatory demands. For natural toxins the design and application of broad specificity antibodies on multiplex and microarray platforms using a single combinational sample preparation offers this opportunity. High quality broad specificity antibodies to the toxin targets (microcystins; cylindrospermopsin, saxitoxin, domoic acid, okadaic acid, brevetoxin) were produced and fully characterised with regards sensitivity and cross reactivity. A planar waveguide immunoassay platform for the multiplex detection of the three key regulated groups of marine toxins and two cyanobacterial toxins also indicated to be an emerging issue in seafood has been developed. Toxin-protein conjugates were spotted onto sensor slides and molecular interactions between antibodies and conjugates were measured using secondary antibodies labelled with a fluorescent dye. The assays were optimised with regards sensitivity by using a chequer board of dilutions of the key reagents. The speed of the assay was optimised from 45 min, by studying the reaction kinetics, until a fully completed test could be performed within 15 minutes. The sensitivity (IC50) for each toxin group has been illustrated as 0.06, 0.42, 1.86, 1.40 and 0.19 ng/mL for saxitoxin, okadaic acid, domoic acid, microcystins and cylindrospermopsin in water samples. The assay demonstrates high suitability for toxin detection in seafood samples.

Keywords: *Marine toxins, microcystin, cylindrospermopsin, microarray, biosensor*

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L-64
AMBIENT MASS SPECTROMETRY: A TUTORIAL

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The introduction of desorption electrospray ionization mass spectrometry (DESI MS) by Cooks and coworkers in 2004 brought, for the first time, widespread attention to the concept of open air surface analysis under ambient conditions. Contemporary with the disclosure of DESI, work carried in parallel by other research teams explored a similar philosophy in chemical analysis. Examples include the patent on the ion source named Direct Analysis in Real Time (DART) filed in December 2003, Shiea's work on open air laser based ion sources, and work by the Van Berkel group at Oak Ridge National Laboratory on surface sampling probes (SSPs) for direct sampling of thin layer chromatography plates first published in 2002. DESI, DART, and other ambient MS techniques enabled an exciting new perspective on ways to perform both qualitative and quantitative chemical investigations on samples not typically amenable to direct MS analysis. As a bonus, direct analysis on nativ e surfaces could be done, in most cases, without sample preparation. The field of ambient MS was thus born, reigniting the interest in finding new ways of making ions for posterior mass spectrometric analysis. Our group and others have classified ambient MS techniques based on their intrinsic desorption/ionization mechanisms. The sub-divisions that we propose are as follows:
(1) one-step techniques where desorption occurs by solid-liquid extraction followed by ESI, APPI, sonic spray, or CI ion production mechanisms;
(2) one-step plasma-based techniques involving thermal or chemical sputtering neutral desorption followed by gas-phase chemical ionization;
(3) two-step techniques involving thermal desorption or mechanical ablation in the first step followed by a second, separate step where secondary ionization occurs;
(4) two-step techniques involving laser desorption/ablation followed by an independent secondary ionization step;
(5) two-step methods involving acoustic desorption approaches;
(6) multimode techniques combining two or more ambient MS techniques;
(7) one-of-a-kind techniques that make use of other principles for desorption or ionization which do not belong to any of the previous categories. In this presentation we will provide an overview of each of these classes of ambient MS techniques, with an emphasis on the strengths and weaknesses that each one may have for different types of applications.

Keywords: *Ambient mass spectrometry, tutorial*

Acknowledgement: *NSF/NASA*

L-65
ACCURATE MASS FRAGMENT LIBRARY FOR RAPID SCREENING FOR PESTICIDES ON THE SURFACE OF IMPORTED PRODUCE USING AMBIENT PRESSURE DESORPTION IONIZATION WITH HIGH-RESOLUTION MASS SPECTROMETRY

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U.S. food and agricultural imports have been increasing steadily for decades, intensifying the need for a rapid, sensitive, and cost-effective screening technique to ensure that the food supply is safe. A method has been developed that uses polyurethane foam disks to sample the surface of a variety of incoming produce. The foam disks are then directly analyzed using transmission-mode direct analysis in real time (DART) ambient pressure desorption ionization coupled to a high-resolution Orbitrap Exactive. Now a library of accurate mass fragments and isotopes has been built using an Orbitrap Q-Exactive mass spectrometer to provide more confidence in the identification of the pesticides detected. The Q-Exactive has a quadrupole mass filter, providing the capability of data dependent fragmentation. A temperature gradient for the DART helium stream and multiple collision energies were employed to detect and fragment over a hundred pesticides of varying chemical classes, sizes, and polarities. The accurate mass capabilities of both precursor and fragment ions of the instruments is essential in correctly identifying chemical contaminants on the surface of imported food items. Additionally, including isotopes in the database offers another tool in the identification and confirmation process. The commodities investigated range from smooth skinned produce such as apples to rougher surfaces like broccoli. The minimal sample preparation and lack of chromatography has shortened the analysis time significantly and the simplicity and robustness of the technique make it ideal for rapid screening.

Keywords: *DART, Pesticides, Produce, Rapid Screening*

L-66
DIRECT FRUIT PEEL ANALYSIS BY DART–ORBITRAP–MS^n

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In this presentation the study of xenobiotics presents in fruits peel by exposing it (without any pre-treatment) to a high-resolution orbitrap direct analysis real time mass spectrometer (DART–HRMS) will be reported. Variables including DART gas heater temperature, and pressure, source-to-MS distance and sample velocity were investigated. The duration of one sample analysis by DART–MS analysis lasted ca. 1 min, and benefits of both, high-resolution and tandem mass spectrometry, to elucidate non-target or unknown compounds. Identification of post-harvest fungicides, antioxidants and sugars in fruit peel was performed in the positive ion mode. The lowest imazalil concentration that could be detected by the proposed approach was 1 ng, with a dynamic range from 1 to 2,500 ng (equivalent to a concentration of ca. 300 µg/kg), which is well-below the maximum residue limit. For oranges and apples, direct peel exposition demonstrated good between-in day precision results (within 20 % for any concentration) and proper linearity R^2 0.99 for apple. A comparison of the results obtained using the direct peel screening DART-based method was made with those obtained by DART analysis of solvent extracts as well as those obtained by injecting these extracts by Ultra High Performance Liquid Chromatography Orbitrap Mass Spectrometry (UHPLC–Orbitrap). The results are in good agreement. Thus, the proposed method proved to be quantitatively accurate with indisputable identification specificity. As an independent method, the approach of direct scanning of peel is of high interest and of potential future within food analysis to guarantee safety, quality and authenticity.

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Keywords: *DART–Orbitrap-MSn, xenobiotics,UHPLC–Orbitrap*

L-67
RAPID WINE PROFILING STRATEGIES: EXPLORING AN AMBIENT IONIZATION METABOLOMICS APPROACH COUPLED WITH EXTRACTION TECHNIQUES

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Fingerprinting and profiling of wines using mass spectrometric (MS) approaches are fundamental for authentication and characterization of wines. A robust analytical approach combining ambient ionization with tandem high-resolution mass spectrometry was developed to rapidly identify marker compounds and classify varietal species. Wine fingerprinting can be assessed with this method by directly measuring the wines without any sample pre-treatment to distinguish between red and white wines. Selective wine profiling using phenolic characterization was carried out by a targeted liquid-liquid extraction employing ethyl acetate to further determine the varietal grape species used in the production of red and white wines. The liquid extracts were subjected to both high resolution mass spectrometry (HRMS) LC-MS (Information Dependent Acquisition (IDA)) and Direct Analysis in Real Time (DART) MS analysis generating individual profiles for over 150 wine samples. Comparing the DART-MS approach with the traditional LC-MS method, increased sample throughput was achieved and alternatively direct coupling of DART with an automated solvent-free extraction employing solid phase micro-extraction (SPME) sorbents, including polystyrene-divinylbenzene-polyacrylonitrile (PS-DVB_PAN), C18-polyacrylonitrile (C-18-PAN), phenylboronic acid-polyacrylonitrile (PBA-PAN) and a mixed-mode phase (C18+ benzenesulfonic acid cation exchanger) a direct wine profiling approach was developed. The SPME ambient MS workflow demonstrates a novel approach for wine analysis with rapid all-in-one analyte extraction and desorption yielding complementary selective profiles as compared to liquid-liquid extraction. Data interpretation was handled using principal component analysis (PCA), generating scores plots clearly differentiating several red and white wine varieties.

Keywords: *Metabolomics, Wine, Profiling, Ambient MS, SPME*

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L-68
NOVEL MS TECHNIQUES FOR THE ANALYSIS OF FOOD AND FOOD CONTACT MATERIALS

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Mass spectrometry (MS) either as stand-alone technique or in combination with separation sciences is one of the fastest developing techniques in instrumental analytical chemistry. Within the wide field of applications of MS, food related topics (analysis of foods, beverages or feeds) as well as any type of food contact materials (materials for processing or packaging) play an eminent role. MS is a perfect tool for the evaluation of food quality (using specific marker substances) but also for food adulteration or contamination. In this presentation an overview of recent developments in the fields of MS with respect to the analysis of foods and food related materials will be given. A special emphasis will also be set on the analysis of plastic materials commonly used in food packaging [1]. Two different strategies will be compared namely direct analysis by MS with only minimum sample preparation and the use of MS as a detector for separation techniques involving chromatographic separation and in many cases also other sample pre-treatment steps prior to MS analysis. Whereby the first approach seems the perfect tool for fast screening of large batches of samples it often can only provide qualitative to semi-quantitative results. When it comes to accurate quantitative analysis, sample pre-treatment and in many cases chromatographic separation is unavoidable. MS techniques dealt with will include several representatives of the relatively young field of ambient MS such as direct analysis in real time (DART) and desorption electrospray (DESI) as well as the even younger direct spray methods such as thin layer chromatography (TLC) spray [2] a technique just recently developed by our group. Besides these, also the more traditional use of MS as a detector for (particularly) liquid phase separations will be discussed. Thereby a special emphasis will be given to the different ionization techniques and their strong- and weak points. Within this issue a number of selected application in combination with the analysis of foods and food related materials will be provided.

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Keywords: *Mass spectrometry, Direct spray techniques, Ambient mass spectrometry, Food analysis, Food contact materials*

L-69
RECENT ADVANCES AND CHALLENGES OF AMBIENT IONIZATION MASS SPECTROMETRY IN FOOD ANALYSIS

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Quality and safety throughout the food production chain depends on the availability of rapid screening methods for ingredients and contaminants. Those methods should be able to address both the expected compounds as well as the unexpected. The latter might originate from natural sources, (for example toxins), from fraud (for example melamine) or from illegal production methods (for example hormones). High sampling and analysis frequencies at low costs are desired. But such a wide scope of analytes, present at different levels and in complex food matrices, can only be covered by mass spectrometry (MS). Moreover, the combination of gas chromatography (GC) or liquid chromatography (LC) with MS is rather slow and costly. Therefore, rapid screening in food analysis is often limited to a few analytes only and not performed by MS but by immunoassays. In the nineties following the invention of triple quadrupole tandem mass spectrometry (MS/MS) several papers and presentations dealt with the so-called 'dilute-and-shoot approach.' Instead of an LC column samples were injected in flow injection mode and instantaneous MS results were obtained. Soon the sample matrix-dependent ionization suppression phenomenon was observed, precluding quantitative and robust analysis results. Since 2005 different modes of ambient ionization MS have been introduced and again the promise of reliable data in seconds tempted several researchers to investigate the applicability and pitfalls of 'instantaneous' analysis. The key question is whether or not these techniques are reliable enough for rapid screening in food analysis. Critical issues are ease-of-use, sample handling (if any), reproducibility, data handling, etc.
Desorption electrospray ionization (DESI), plant spray ionization, direct analysis in real time (DART) and laser ablation electrospray ionization (LAESI) coupled to linear ion trap, orbitrap and/or ion mobility quadrupole time-of-flight MS were explored in different food analysis applications.
In this presentation an overview will be given of recent achievements and the challenges encountered. In general ambient ionization MS seems to be less sensitive than GC/MS or LC/MS but in many agrifood applications it is realistic to perform a qualitative or semi-quantitative rapid (pre)screening using ambient MS following a minimum sample preparation.

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L-70
LESA ANALYSIS OF BACTERIAL SURFACE: FROM MICROBIAL COMMUNICATION TO FOOD SAFETY

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Fast screening of surfaces was recently realized by a combination of Liquid Extraction Surface Analysis (LESA), automated chip-based nanoelectrospray ionization, and high-resolution mass or tandem mass spectrometry using LTQ-Orbitrap XL. This method was recently extensively explored for study chemistries on surface of leaves or floral petal (Arabidopsis thaliana, Iceland poppy) and to discover allelochemicals involved in co-cultivated fungi and bacteria strains. Further this method was tested to follow bacterial and fungal food contamination.

Keywords: *LESA, MS, bacterial and fungal food contamination*

L-71
DART–MS: AN OVERVIEW OF CONCEIVABLE APPLICATIONS IN FOOD ANALYSIS AND AUTHENTICATION

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Since its introduction approx. 8 years ago, ambient mass spectrometry (AMS) employing Direct Analysis in Real Time (DART) ion source coupled with spectrometry (MS) has been becoming a commonly used technique in modern laboratories. DART is APCI-related technique producing relatively simple mass spectra, dominated by protonated molecules $[M+H]^+$ in positive-ion mode, or deprotonated molecules $[M-H]^-$ in negative-ion mode. The addition of ammonia or other "dopants" to the DART gas stream can be used to form single-charge adducts such as $[M+NH_4]^+$ or $[M+Cl]^-$ for compounds that would not readily form molecular ions or protonated molecules. However, due to the absence of sample separation, strong matrix effects, typically ion suppression, may limit detection of minor components. On the other hand, minimal sample preparation, together with high throughput potential, are the major benefits offered by DART-MS. In this presentation, the overview of our five years experience with DART-MS the most challenging applications will be shown:

- Target analysis – quantitative analysis of food contaminants
- Authentication of food samples based on DART-MS fingerprints / profiles
- Characterization of organic nanoparticles
- Examination of packaging materials for potential migrants

L-72
HYPERSPECTRAL LINE-SCAN RAMAN CHEMICAL AND NIR IMAGING TECHNOLOGIES FOR FOOD ADULTERANT DETECTION AND INGREDIENT AUTHENTICATION

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The Environmental Microbial and Food Safety Laboratory, ARS, USDA, is one of the leading groups for the development of spectral imaging technologies and methodologies for food safety and quality evaluation. The spectral imaging technologies include Raman, fluorescence, and visible/near-infrared (NIR) reflectance for hyperspectral and multispectral applications, and can also deliver high-speed online inspection of food and agricultural products on high-throughput processing lines. Most recently, a line-scan-based hyperspectral Raman chemical imaging system was developed that uses a high-intensity 785 nm line laser as an excitation source to generate Raman scattering signals across a sample. Spatially-resolved (laser line) Raman scattering signals (e.g., 1024 Raman spectra for 1024 pixels) across the sample (a 200-mm-wide field of view) are simultaneously collected per line-scan. The rapid data acquisition time allows imaging of large sample areas—a speed improvement of at least 3 orders of magnitude compared to conventional Raman imaging approaches. In this presentation, we present our line-scan Raman and NIR imaging systems that can be used to quickly and accurately inspect bulk powder foods, such as milk powder contaminated with low concentration of melamine. Due to its high speed and allowance for larger sample areas, the line-scan imaging format enables implementation of Raman chemical and NIR methods for screening/sampling of larger amounts of food products for low-concentration contaminants in a way that is useful to contaminant detection and ingredient authentication applications for the food industry. For detecting low-concentration contaminants, the limit of detection and the limit of quantification can be vastly improved by using spatially-resolved spectral image analysis methods, over conventional point-source spectroscopic detection methods, to rapidly map large sample areas. The line-scan Raman and NIR imaging methods have the potential to narrow the detection gap between traditional conventional spectroscopy-based methods that can detect higher concentrations of contaminants/adulterants and traditional analytical methods that can detect trace amounts. Future implementation of the methods presented here, for in-plant screening of food ingredients, may provide food processors with a critical tool for food safety and authentication that benefits food industries and consumers worldwide.

Keywords: *Food authentication, adulterants detection, Raman, hyperspectral, spectral imaging*

L-73
EARLY CONTAMINANT DETECTION IN FEED MILLS: LESSONS FROM THE MELAMINE CRISIS AND TEST OF INNOVATIVE STRATEGY FOR ON-LINE CONTROL

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Different national/international authorities invest in research programs to improve testing methods in order to avoid further problems issued from food and feed contaminations. This initiative has been boosted by recent crises such as the melamine scandals in pet and human food commodities in 2007 and 2009, respectively. Milk powder contaminated sickened and killed a certain number of babies and the contaminated wheat gluten and chicken feed caused enormous economic losses to the industry. Accurate, rapid and sensitive analytical methods are needed for the assessment of the quality and safety of food and feed products. These methods should be directly implemented at the entrance of the factories/industries preventing the contamination of the whole production chain. The main goal of this study is to develop an alternative procedure based on Near Infrared (NIR) spectroscopy and chemometrics for characterizing typical feed ingredients and detecting the possible presence of contaminants from various types at the entrance of the production chain. Molecular spectroscopy knowledge about the feed ingredients combined with pattern recognition techniques and multivariate regression models led to the creation of decision rules. These rules allow checking compliance against specifications and deciding whether to reject or accept the product. The complete procedure has been developed and validated at laboratory level. Then, it has been adapted for implementation in a large feed mill in order to detect anomalies due to an accidental or fraudulent addition of contaminant or no authorized additives. In this work, all the efforts have been put on the setting of multivariate specifications for the NIR spectroscopic characterization of pure soybean meal. The proposed procedure can be used with any other vibrational spectroscopic data (e.g. MIR or Raman) and possible feed ingredient or compound feed. The methodology has been tested in a feed mill where different trucks containing the soybean meal were deliberated contaminated. The c ontamination has been done with whey and DDGS (Dried Distillers Grains with Solubles) during the loading at the entrance of the production chain. A sampling system allowed getting samples that were analyzed using an at-line NIR spectrometer. The spectra were submitted to the decision rules to characterize soybean meal materials and to detect the adulterated samples. The results demonstrated the possibility to implement fast and efficient control strategy to detect on-line contamination (whatever the origin) at the discarding place.

Keywords: *Contaminants, soybean, industry, spectroscopy, chemometrics*

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L-74*
COMPREHENSIVE ACCURATE MASS QUANTIFICATION OF VETERINARY DRUGS AND N-ADULTERANTS IN ANIMAL COMPOUND FEED AND FEED MATERIAL BY LIQUID CHROMATOGRAPHY COUPLED TO ORBITRAP MASS SPECTROMETRY

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Livestock production has become an important economic activity worldwide. Animal feeding stuffs do not only require sufficient quality from a nutritional point of view but also safety which means ensuring the absence of contaminants and residues. These substances can enter the feed via different paths and further pass through to the consumers via the food chain. In consequence, the use of all veterinary drugs as feed additives, other than coccidiostats and histomonostats, has been banned by the European Commission since 2006. Reliable analytical methods are therefore needed to ensure the control of the feed food chain. On the other side N-adulterants (e.g. melamine) have been illegally added as a nitrogen source in feed and food (e.g. pet food, milk and milk products) to apparently enhance the protein content, with severe toxic effects. There is therefore a strong need in Europe for establishing Strategies for Early Quality and Safety Assurance in the Feed Chain. The European FP7 project QSAFFE aims at contributing to such strategies. In order to address such threats, one of the techniques used was the advanced platform of high resolution-orbitrap technology. The high resolving power of the Orbitrap provides precise mass accuracy, resulting in high selectivity which enables qualitative and quantitative analysis in complex matrices. Simple generic sample preparation procedures were applied, including extraction of the samples with a combination of organic solvents. The mass spectrometer was operated in full scan with polarity switching between positive and negative modes, using heated Electrospray Ionization. Due to the complexity of the samples the resolving power proved to be the key for the discrimination between interfering masses from the matrix and the exact masses of the compounds in order to achieve mass accuracy of 5 to 10 ppm. A thoroughly validation study was successfully performed and evaluated in animal compound fee d and Distillers Grains for veterinary drugs and in soyabean meal for melanine and cyanuric acid. The developed methods included a wide range of chemical groups, authorized coccidiostats, banned coccidiostats, macrolides, tetracyclines, nitroimidazoles, amphenicols, quinolones, sulphonamides, tranquilizers, non-steroidal anti-inflammatory drugs and benzimidazoles. A thorough single-laboratory validation procedure was constructed evaluating specificity, sensitivity, linearity, precision and accuracy and showed satisfactory analytical performance for quantification allowing the determination of the compounds at very low concentrations. Additionally, a simplified quantification approach only depending on the response factor was successfully evaluated. The proposed multi-analyte methods proved their rapidness, simplicity, reliability, demonstrating that LC utilized with an orbitrap mass spectrometer is a dominant analytical platform, suitable for official control of residues in complex compound feed and feed material.

Keywords: *Veterinary drugs, Melamine, Animal feed, Feed material, Liquid chromatography-high resolution mass spectrometry*

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L-75*
TRACEABILITY OF DISTILLERS DRIED GRAINS AND SOLUBLES BY STABLE ISOTOPE RATIO MASS SPECTROMETRY

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Distillers Dried Grains and Solubles (DDGS) are a novel feed material mainly used as feedstock for ruminants, poultry, pigs and fish in aquaculture. They are a by-product of the alcohol distilling process obtained by drying solid residues of fermented grains (e.g. corn, wheat, barley), to which pot ale syrup or evaporated spent wash was added [1]. As a result of the upgrowth of the fuel-ethanol industry, DDGS became a global commodity and play an even more important role in the feed market due to their low price and their high nutrient content (proteins, fat) [2]. DDGS were chosen as a commodity to be analyzed as they are increasingly traded amongst different countries and in the production of DDGS (especially from the fuel-ethanol industry) strategies to increase the yield of ethanol could possibly become more relevant than quality issues of DDGS. In addition, the level of an assigned risk might be linked to certain areas of origin and a differentiated level of control might be appropriate, which requires that the origin must be traceable and verifiable, not only on the basis of documentation systems provided by the producer or distributor but also by analysis in the laboratory [3]. In this respect, DDGS from different countries and derived from different botanical raw materials have been analyzed by Stable Isotope Ratio Mass Spectrometry (IRMS) for the isotopes of hydrogen ($^{2}H/^{1}H$), carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{18}O/^{16}O$) and sulfur ($^{34}S/^{32}S$). As expected, analyses showed clear differences in the $\delta^{13}C$ values for DDGS produced from corn (-13.0‰) and wheat (-26.9‰) representing C4 and C3 plants. Investigation of 88 corn DDGS samples from China, the European Union and the United States revealed differences according to the place of origin of the samples, primarily due to their $\delta^{2}H$ (-144.2‰ to -89.1‰) and $\delta^{18}O$ (17.4‰ to 23.2‰) values. As well differences in the $\delta^{15}N$ values (1.17‰ to 5.67‰) were obtained indicating agricultural and/or production practices possibly linked to certain regions. However, isotopic differences of sulfur ($^{34}S/^{32}S$) could only partly be associated with geographical origins. Results of univariate and multivariate analyses of the IRMS data for DDGS from different origins will be presented and the application of IRMS for feed authenticity research is highlighted.

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Keywords: *IRMS, authenticity, feed materials, DDGS*

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L-76
A COMPREHENSIVE APPROACH TO DEFINE THE CHEMICAL BLUEPRINT OF EXTRA VERGIN OLIVE OIL

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Extra virgin olive oil's sensory attributes and in particular specific aroma defects are officially responsible for oil classification or declassification. Such an important issue is managed by official Panels. The aim of the present work is to propose an innovative analytical approach exploiting the informative content of comprehensive multidimensional gas chromatography (GC×GC) coupled to a mass spectrometry (MS) data set to define the chemical blueprint of virgin olive oil volatiles. A series of virgin olive oil samples were evaluated by a Panel test and then analysed in two laboratories, using different instrumentation to cross-validate the entire methodology. A first untargeted approach was applied obtaining low discrimination power. Therefore, peak features were reliably identified (261 compounds) on the basis of the match of the MS spectrum and linear retention indices, and subjected to successive pair-wise comparisons, based on 2D patterns, which revealed peculiar distribution of chemicals correlated with the samples sensory quality. A further step forward was the application of the principle of sensomics based on the odor activity value, thus obtaining much more discriminative results. This approach proved to be a valuable tool to support, or even replace, sensory evaluation.

Keywords: *Sensomics, comprehensive multidimensional gas chromatography (GC×GC), fingerprinting, volatile compounds, olive oil*

L-77
DEVELOPMENT AND OPTIMIZATION OF AN ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–QUADRUPOLE-ORBITRAP MASS SPECTROMETRY METHOD FOR ANALYSIS OF MULTIPLE PHARMACEUTICALS, PLANT TOXINS, AND OTHER SECONDARY METABOLITES IN HERBAL DIETARY SUPPLEMENTS

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The use of herbal dietary supplements has significantly increased in recent decades. In 2010, the sales of these products in the U.S. reached an estimated figure of more than 5.2 billion dollars. One of the main factors supporting this trend is that consumers assume the use of herbal dietary supplements to be a safe and natural alternative to traditional medical treatments. A wide variety of herbal dietary supplements advertised for treatment of diabetes, hypertension, obesity and other common health problems are currently available. Although these supplements do not undergo premarket review the US Food and Drug Administration (FDA) prior to marketing, FDA has the authority to seize products which represent risk to consumers. The major safety and authenticity issues related to herbal dietary supplements include the presence of natural compounds with toxic effects, the presence of synthetic pharmaceuticals added as adulterants, and potential interaction of some natural components with prescription drugs. Accidental substitution of an authentic ingredient with another plant or intentional adulteration with cheaper plant materials may cause serious health problems to consumers due to the presence of toxins. Recently, a number of poisonings by plant toxins from herbal dietary supplements have been reported. Deliberate additions of pharmaceuticals to herbal dietary supplements typically aim at intensifying the claimed biological effects of the product. In the current work, an ultra-high performance–quadrupole-orbitrap mass spectrometry-based method was developed, optimized and validated for simultaneous screening and quantitation of pharmaceuticals and plant toxins/metabolites in various herbal dietary supplements. The target pharmaceuticals included drugs used to treat diabetes, hypertension, and inflammation, as well as weight loss drugs and PDE-5 inhibitors. The plant toxins/metabolites included in the method represented compounds whose occurrence is either characteristic in specific plant family or genus (e.g. ephedra alkaloids or aristolochic acids) or which occur in a wide range of herbs and other botanicals (e.g. pyrrolizidine alkaloids). A QuEChERS-like extraction allowed for effective isolation of the majority of analytes with recoveries ranging from 70 to 120%. The method was found suitable for detection/quantification of both plant toxins/metabolites at µg/kg to mg/kg concentrations and pharmaceuticals at g/kg concentrations. The high resolution spectra recorded in full MS–data dependent MS/MS acquisition mode provided a high degree of confidence in identifying the analytes. The method was used to examine 30 herbal dietary supplements collected in the U.S. The optimization of sample preparation, chromatographic separation and mass spectrometric detection, as well as requirements for confirmation of analyte identity and strategies for quantitation will be discussed in the presentation.

Keywords: *Herbal dietary supplements, pharmaceuticals, plant toxins, adulteration, UHPLC–MS*

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L-78
DETECTION OF UNAUTHORISED GENETICALLY MODIFIED ORGANISMS (GMOs)

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Within Europe a shift can be seen in recent years from the detection of GMOs for the purpose of correct labelling towards the detection of EU-unauthorised GMOs (UGMOs) that have not yet been assessed for their safety for the consumer and the environment. In practice the detection of UGMOs is often performed by a qPCR-based screening strategy for common DNA elements (promoters, coding sequences, terminators, etc.) in different GMOs that may also be present in UGMOs. Unexplained GMO elements may indicate the presence of UGMOs. UGMO identification through sequence analysis should always be the next step when the presence of GMO elements, unexplained by the presence of GMO events in the same sample, leads to the suspicion of UGMO presence. Positive identification of UGMOs should be based on the sequence identification of genetic constructs that contain combinations of GM elements that are not reported for authorized GM constructs, or that are unlikely to occur in the unmodified crop species. Therefore, if unexplained GMO elements are identified, the next step is nowadays a standard Sanger sequencing strategy starting from the single identified GMO element(s). Only when an event-specific qPCR method is available for the UGMO in question a more straightforward approach can be taken. In the laboratory practice, this approach of UGMO detection and identification is an increasingly lengthy and costly procedure, with the numbers of potentially present UGMOs as well as candidate GMO elements steadily growing. New developments in high-throughput sequencing (HTS) seem to offer better options for simultaneous detection and identification of both GMOs and UGMOs in a single sample. In this presentation different HTS strategies, including Illumina HiSeq2000 and PacBio RS, with and without initial GMO element enrichment steps, will be discussed in the light of the increasing global production of GMOs. Also the pros and cons of different HTS platforms will be discussed with relation to UGMO detection and identification.

Keywords: *GMO, UGMO, NGS sequencing, HTS sequencing*

L-79
PREHARVEST MYCOTOXIN CONTAMINATION OF AGRICULTURAL COMMODITIES: CURRENT ISSUES ON DETECTION AND CONTROL

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Aflatoxins, trichothecenes and fumonisins are the major mycotoxins (fungal secondary metabolites) that contaminate crop plants and, as a result, are of great importance to food and feed safety. Aflatoxins are produced mainly by the two *Aspergillus* species, *A. flavus* and *A. parasiticus*, whereas trichothecenes and fumonisin by *Fussarium* species. Regulatory guidelines affecting the permissible levels of these toxins account annually for hundreds of millions of dollar equivalents (internationally) of crop losses, as well as loss of health and human life across the world, affecting agricultural economics, world trade and food security on a global basis. Strong connections between environmental conditions and occurrence of mycotoxins in world crops are implicated in promoting mycotoxin contamination in certain regions of the world from climate change (warming). So many environmental factors affect mycotoxin contamination in crops that predicting outbreaks of m ycotoxin contamination of crops is not well developed. Two main current issues concerning mycotoxins are sampling and accurate, rapid detection of contaminated commodities, as well as effective measures for controlling pre- and post-harvest contamination of crops and commodities. Because of the skewed distribution of contamination in the affected commodities, sampling for detection requires a large number of samples for an accurate assessment. Current testing measures are expensive and time consuming, so testing large number of samples is prohibitive. New methods (such as biosensors and imaging techniques) are being developed that overcome these hurdles and will potentially provide easy and rapid testing procedures at relatively lower costs. Also, now for the first time control measures for this problem appear within reach. For practical and sustainable control of pre-harvest mycotoxin contamination to be realized, information is being rapidly gathered about the fungus, the affected crops and the specific molecular factors (both in the plant and the fungus) involved during host plant-fungus interaction. Use of novel tools such as genomics, proteomics and metabolomics is providing us with the best and the quickest opportunity to achieve a clear understanding of the survival of toxigenic fungi in the field, the ability of the fungus to invade crops, and the process of toxin contamination under various environmental conditions. Significant progress has been made recently in understanding the genomic makeup of the toxigenic fungi as well as in the study of host crop resistance to fungal invasion through the use of proteomics. In this presentation, the use of contemporary methods and tools in deriving the requisite information for developing effective strategies to interrupt the machinery in the fungus for production of these toxins, as well as to enhance host-resistance against fungal invasion and toxin contamination of crops will be discussed.

Keywords: *Mycotoxin, crops, food security, contamination, fungi*

L-80
"MASKED" MYCOTOXIN DETECTION: WHAT IS A POOR CHEMIST TO DO?

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Novel fungal toxins and metabolites of known toxins that have not been amenable to detection using established reference methods are routinely termed “masked” mycotoxins. Typically these are metabolites that are structurally related to mycotoxins of economic importance, but which are not observed directly during screening of commodities or foods. For example, many of the mycotoxins form glucoside conjugates. Aside from concern about the toxicity of the compounds themselves, there is concern about the possibility that they may serve as reservoirs for the parent toxin. With the recent widespread application of liquid chromatography-mass spectrometry, in particular the application of highly selective mass detectors, detection of masked mycotoxins has become more feasible. This presents a new set of issues for analysts, not the least of which is nomenclature (is a masked mycotoxin really “masked” if you can detect it?). Functionally the issues are more significant: given limited resources, which of these metabolites are worth detecting and quantifying? The ideal detection system would measure the desired analytes in a cost effective manner that supports monitoring, rather than impedes it. For good reason analysts generally have greater confidence in methods that directly detect toxins, based on physical or chemical properties, rather than those that indirectly detect toxins, such as immunoassays. However, field applications of direct detection techniques still face significant obstacles. Conversely, the mechanisms for making field applications from immunoassays are well established. Such assays can be inexpensive to produce and require minimal training for operation, which are significant advantages for screening applications. However, when it comes to detecting masked mycotoxins the immunoassays have their own set of limitations. One of the major issues for immunoassays is whether the antibodies directed against the parent toxins (which are the basis for screening kits) are able to bind with, and detect, the metabolites of interest. Trichothecene mycotoxins, such as T-2 toxin, are common contaminants of commodities and foodstuffs. Recently the glucosides of T-2 toxin and related HT-2 toxin were discovered in fungal cultures and their presence was detected in wheat and oats. In order to improve the isolation and detection of T-2 and HT-2 glucosides, several antibodies directed against these masked mycotoxins were developed and applied in a biosensor for detecting them in wheat. The strong cross-reactivity of several of these antibodies with both T-2 toxin and its glucoside suggests that detection of the two analytes together, as a combined response, may be feasible. This is a first step in addressing the issue of the simultaneous detection of masked mycotoxins, along with their parent toxins, in foodstuffs using rapid immunoassays.

Keywords: *Masked mycotoxin, T-2 toxin, detection, immunoassay, biosensor*

L-81
QUANTITATION OF AFLATOXINS FROM CORN AND OTHER FOOD RELATED MATERIALS BY DIRECT ANALYSIS IN REAL TIME –MASS SPECTROMETRY (DART–MS)

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Ambient ionization coupled to mass spectrometry continues to be applied to new analytical problems, facilitating the rapid and convenient analysis of a variety of analytes. Recently, demonstrations of ambient ionization mass spectrometry applied to quantitative analysis of mycotoxins have been shown. Direct analysis in real time (DART) ionization is an ambient ionization technique that has shown great potential in the analysis of mycotoxins. In this study, DART ionization coupled to a high resolution mass spectrometer (MS) was used for screening of aflatoxins from a variety of surfaces and the rapid quantitative analysis of aflatoxins extracted from corn and other food related matrices. Sample preparation procedure and instrument parameter settings were optimized to obtain sensitive and accurate determination of aflatoxins. 84:16 acetonitrile / water extracts of corn deposited on paper substrate were analyzed by DART–MS. The lowest calibration level (LCL) f or aflatoxin AFB1 was 4 µg/kg. Quantitative analysis was performed with the use of matrix-matched standards employing the ^{13}C-labeled internal standard for AFB1. DART-MS of spiked corn extracts gave linear response of the range 4–1000 µg/kg. Good recoveries (94–110%) and repeatabilities (RSD 0.7–6.9%) were obtained at spiking levels of 20 and 100 µg/kg with use of an isotope dilution technique. Trueness of data obtained for AFB1 in maize by DART-MS was demonstrated by analysis of corn certified reference materials. Broad applicability of the DART–MS from paper substrate technique was demonstrated by adaptation to aflatoxin extracts from other food related matrices.

Keywords: *Mycotoxins, aflatoxin, mass spectrometry, direct analysis in real time (DART)*

L-82
HEXABROMOCYCLODODECANE AS A PROTOTYPE OF THE ANALYTICAL CHALLENGES OF EMERGING FLAME RETARDANTS IN FOODS

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Exposure to many persistent organic pollutants (POPs) occurs via our food, e.g. polychlorinated dibenzodioxins. Some flame retardants (FRs), like polybrominated diphenyl ethers (PBDEs), are listed as POPs, and others, like hexabromocyclododecane (HBCD) are candidate POPs. Legacy POPs are typically purified from environmental and food matrices and analyzed by high resolution gas chromatography-mass spectrometry (HRGC/MS) by long-established methods. HBCD presents the analyst with a broad set of unique challenges to its quantification in food. We have conducted ADME studies with each of the three HBCD stereoisomers in rats. This presentation will elucidate some of the intriguing properties of HBCD, which are shared by some emerging FRs, and explain how these properties will need to be addressed to analyze emerging FRs in food samples. ADME studies demonstrated that >74% of each HBCD stereoisomer was absorbed following a single oral dose. The isomers were readily eliminated in feces and urine in an isomer-specific manner (β > γ > α). The highest terminal tissue concentrations were in adipose tissue, GI tract, skin and liver, and within these tissues α-HBCD was more concentrated (approximately 100:11:27 α/β/γ). Metabolites were characterized by LC/MS (ESI-) and were distinct for each stereoisomer; hydroxylation for α, debromination for β, and a combination of hydroxylation-debromination-dehydrogenation for γ-HBCD. In addition, 4% of the γ-HBCD dose was stereoisomerized to α-HBCD. Due to multiple chiral centers, HBCD enantiomers were analyzed in tissues and a slight tissue-specific enantioselectivity was indicated. Consistent with the dramatic shift in stereoisomer patterns observed between biota/food (predominantly α-HBCD) and the technical product (γ-HBCD), our data indicated that this shift could be explained by differences in metabolism, elimination, and isomerization among the stereoisomers. Data will also be presented on the mammalian bioavailability of HBCD stereoisomers from household dust at chronic, environmentally-relevant feeding levels. Thus, research has demonstrated that despite being cycloaliphatic, isomer-specific HBCD data cannot be obtained by HRGC/MS due to degradation of HBCD isomers at temperatures above 160°C. This results in quantitation errors and makes LC/MS the preferred method of analysis. These conditions are similar to TBECH, an emerging FR, which also occurs as a family of temperature-sensitive stereoisomers. However, LC/MS conditions are prone to suffer from moderate-to-severe matrix effects, which places a premium on sample preparation and chromatographic performance during food analyses, and will be needed when analyzing phenolic FRs such as TBBP-A and 2,4,6-TNP. Additional analytical issues raised by HBCD enantiomers, stereoisomerization and metabolic markers will also be discussed.

Keywords: *Flame retardants, hexabromocyclododecane, HBCD, LC/MS, metabolism*

L-83
DIOXIN, FURAN, PCB, AND PBDE LEVELS IN U.S. FOODS: SURVEY TRENDS AND CONSUMER EXPOSURE

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The U. S. Department of Agriculture, Agricultural Research Service (USDA–ARS) and Food Safety and Inspection Service (USDA–FSIS) have conducted statistical surveys for dioxins (PCDDs, PCDFs, and PCBs) and polybrominated diphenyl ethers every 5 years since the mid-1990s (mid-1990s, 2002–3, 2007–8). In 2012-13 another survey is being conducted. From these data, background levels of dioxins in domestic beef, pork, and poultry are determined. Due to the structure of the surveys, temporal trends can be tracked and sources of dioxins in the food supply can be investigated. From the mid-1990s to 2008, PCDD, PCDF, and PCB levels in beef, pork and poultry have either declined or remained static. The current survey data will also be compared to past results to determine temporal trends. Animal food products may also contain PBDEs, however most of the available data on food-animal sources of PBDEs are from small market basket surveys and do not provide insights into long-term trends. Since the 2002–3 survey, levels of PBDEs in U.S. beef, pork, and poultry have also been analyzed to provide evidence of, or to refute, the effectiveness of the phase out of PentaBDE and OctaBDE production. In 2012, the Environmental Protection Agency released their chronic oral reference dose (RfD) for 2,3,7,8-TCDD of 0.7pg/kg-day for human exposure. Survey data, along with consumption data for beef, pork, and poultry, are used to calculate consumer exposure estimates to PCDDs, PCDFs, PCBs, and PBDEs. The calculations were also used to determine dioxin trigger levels (2 pg TEQ/g fat for beef and 4pg TEQ/g fat for poultry and pork) used for the survey that would initiate on-farm investigations by FDA for samples above the trigger levels. Currently no poultry or pork samples have been found above trigger levels. There have been 5 beef samples found to be above the trigger level of 2ppt and are under investigation by the FDA. Current beef, pork, chicken and turkey dioxin levels are 0.088–6.46 pg TEQ/g fat, 0.051–0.36 pg TEQ/g fat, 0.046–0.39 pg TEQ/g fat, and 0.052–1.32 pg TEQ/g fat, respectively. Comparing the medians of these data to previous surveys, there are decreases in the overall levels of dioxins over the past 5 years.

Keywords: *Dioxins, PCBs, exposure, PBDEs, U.S. Foods*

L-84
MULTI-CLASS, MULTI-RESIDUE ANALYSIS OF ENVIRONMENTAL CONTAMINANTS AND PESTICIDES IN FISH USING FAST, LOW-PRESSURE GAS CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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A simple and rapid method for simultaneous determination of 193 targeted residues in fish was developed and evaluated to monitor 143 pesticides and 50 environmental contaminants (including the persistent organic pollutants: polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and novel flame retardants). Sample preparation was based on the QuEChERS (quick, easy, cheap, effective, rugged, safe) approach using acetonitrile for extraction and dispersive solid-phase extraction (d-SPE) clean-up with a zirconium-based sorbent. The method was evaluated at four spiking levels and further validated by analysis of NIST Standard Reference Materials (SRMs) 1974b and 1947. By using isotopically-labeled internal standards, recoveries of most analytes were 70–120% with relative standard deviations less than 20% (n=5), even at low spiking levels. The measured values for both SRMs agreed with certified/reference values (71–119% accuracy) for the majority of analytes. The detection limits were 0.1–0.5 ng/g for PCBs, 0.5–10 ng/g for PBDEs, 0.5–5 ng/g for pesticides and PAHs, and 1–10 ng/g for flame retardants, making the method applicable for analysis at environmentally relevant concentrations. The calculated sample preparation cost is less than $3/sample using bulk materials, and small amounts of organic solvents used for extraction reduces solvent waste and environmental impact. Sample preparation for a batch of 10 homogenized samples takes approximately 1 hour per chemist. Fast, low pressure vacuum outlet gas chromatography tandem mass spectrometry (LP–GC/MS–MS) provides fast separation of over 200 analytes in 10 min to achieve high throughput. The developed method was applied for analysis of catfish samples from the market, and residues of pesticides, PAHs and flame retardants were measured in catfish. Furthermore, the results from the new method compared well with the traditional method based on pressurized fluid extraction, gel permeation chromatography, and solid-phase extraction clean-up sample preparation and conventional GC-MS for the analysis of wreckfish samples. The developed method allows analysis of a large number of pesticides combined with novel flame retardants and other legacy plus emerging classes of environmental contaminants from one sample. The generated data on occurrence of these contaminants may advance the understanding of the potential risk posed by these chemicals and aid in future risk assessment and regulations.

Keywords: *Pesticides, environmental contaminants, QuEChERS sample preparation, fast gas chromatography tandem mass spectrometry, catfish analysis*

L-85
ESTABLISHMENT AND APPLICATION OF A ROBUST MASS SPECTRAL LIBRARY FOR SCREENING POTENTIAL CONTAMINANTS IN FEED

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National monitor plans have been launched in many countries for routine survey of contaminants in food. For instant, Chinese MOA issued annual plans to monitor contaminants in animal borne food and feed. Through the targeted contaminants are changed year by year, they are quite limited to compared to potential contaminants, especially for abuse drugs and additives. There is a growing interest from researchers to screen for and identify non-targeted compounds in food, including metabolites and degradates, but also completely unexpected contaminants. Several versions of LC/MS/MS instrument and tools have being developed to face the challenging task. The detected compounds are identified based on empirical calculation of the molecular formula, interpretation of MS/MS fragments and mass spectral library searching. The acquired high resolution and accurate mass spectra are further used to quantify the amounts of detected and identified pollutants in a large set of practical food samples. 100 samples of finished pig feed were collected in nationwide areas with higher price or doubtful advertisement. AB TripleTOF™5000 LC/MS/MS system was used to screen for unexpected feed additives. LC-MS/MS data is processed using principal components analysis (PCA) to find combinations of variables, in this case based on retention times, mass signals and intensities, that explain most of the variance present in a data set. Every sample has a score and every variable has a loading for each principal component, scores and loadings of two or three principal components are plotted in 2D or 3D to visualize analytical results. The scores plot clearly differentiates samples originating from different sample types. Some feeds arrange in the top part of the scores plot. The corresponding loading plot assists to find characteristic marker ions for this grouping. The loading plot indicates mass 254.5 Da at 12.6 min. The high resolution and accurate mass spectra of the detected molecular ion and the automatically collected MS/MS spectrum were further processed using the Formula Finder of PeakView™Software to calculate empirical formulas for the molecular ion and detected fragment ions. Accurate mass information, the isotopic pattern and logic on distribution of chemical elements were used. The information gained from data processing was used to characterize the structure of the identified contaminant and identify the molecule as Dihydropyridine (1,4-dihydro-3,5-dicarbetboxy-2,6- dimethyl-pyridine, 1,4-Dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid diethyl ester). After identification of the additive, a quantitation method of detecting dihydropyridine in feed was developed with an analytical standard by LC–MS/MS with SIM mode. 13 of 100 collected samples were detected for this additive with level from 120ppm to 278ppm.

Keywords: *Non targeted contaminant, LC–MS/MS, feed, library*

L-86
BUILDING A FOOD CONTAMINATION EARLY WARNING SYSTEM WITH UPLC–TOF–MS

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Globalisation of food trade is responsible for emerging risks in food and feed products. Differences in quality standards and regulatory controls among countries potentially lead to accidental and/or intentional contamination of end-products. In order to protect consumer health and the food industry's reputation, it is necessary to control the whole food supply chain, from raw materials to end-products. At present, food analyses are based on targeted methods and are not able to address fraud or adulteration issues or tackle unlisted contaminations. Recent developments in analytical methods have raised the possibility of using non-targeted fingerprinting methods to ensure food and feed safety. Agrifood GPS (Global Protection System) is a 5-year project. It started in January 2012, partly financed by the BPI (Banque Publique d'Investissement, ex-OSEO), a French public-sector institution that provides support and funding for innovation. The focus of the project is global analytical method development to create an early warning system, highlighting non-compliance or unwanted contamination, and providing information that will help a company accept or reject a batch. This approach is based on a metabolomics approach, first developed in the health sector, the aim of which is to highlight chemical markers of an observed effect, such as a difference between population groups, using statistical data analysis. The project applies global methods to various food matrices. Using statistical tools, each new sample is compared with an existing database to check whether or not it falls within the range of the statistical model and if it contains any known contaminants. If the sample is not compliant with the model, the method makes it possible to highlight markers responsible for its differentiation from the existing database. Apple juice matrices analysed with UPLC–TOF–MS (Ultra High Performance Liquid Chromatography – Time of Flight – Mass Spectrometry) are presented as an example of database building. The challenge is to optimize data pre-treatment (retention time alignment, table peak extraction, normalization) to reduce analytical bias in order to keep only relevant information. The data are then processed to create a statistical model, the starting point for the early warning system to address food and feed integrity.

Keywords: *UPLC–TOF–MS, non-targeted analysis, food database, warning system, food safety*

L-87
APPLICATION OF APGC–MS/MS FOR THE DETERMINATION OF PCDD/Fs AND PCBs IN FEED AND FOOD MATRICES

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The network of European Union Reference Laboratory (EU-RL) and National Reference Laboratories (NRLs) of EU Member States for Dioxins and PCBs in Feed and Food developed analytical criteria for the application of GC–MS/MS as confirmatory method for determination of PCDD/Fs and DL-PCBs. These criteria were based on experiences with GC–MS/MS systems in electron ionization (EI) mode. Recently also MS/MS systems equipped with an atmospheric pressure GC source (APGC) showed sufficient sensitivity for determination of PCDD/Fs and DL-PCBs at low concentrations in food and feed samples. The soft ionisation at atmospheric pressure in N2 plasma under charge transfer conditions can increase the intensity of the monitored molecular ion of PCDD/Fs and DL-PCBs and reduce the fragmentation in the ion source. Especially for the low maximum and action levels applicable for PCDD/Fs in feed and food, low LOQs for the individual congeners are of importance. The APGC–MS/MS measurements were performed using an APGC Xevo TQ-S provided by Waters, Manchester, UK, at the Waters application laboratory in Manchester using standards and samples provided by the EU-RL and RIKILT, and at the EU-RL in Freiburg. The extraction and clean-up processes for food and feed samples for APGC–MS/MS were the same as for GC–HRMS. For comparison the identical extracts were measured on APGC–MS/MS and GC–HRMS. Additionally a modified faster clean-up with a reduced number of clean-up steps was applied. Results of APGC–MS/MS measurements were also compared with assigned values from PTs and mean values of quality control materials obtained from GC-HRMS analysis. The APGC–MS/MS system showed sufficient sensitivity to analyse samples in the range of action and maximum levels. Deviations of the results of APGC-MS/MS from reference values were below 20 % for the WHO–PCDD/F–TEQ. Also deviations for individual congeners were in an acceptable range. For the reduced clean-up considerable differences between the assigned value and the APGC–MS/MS result could be observed due to unresolved interferences on PCDFs. First experiences showed sufficient sensitivity of the APGC-MS/MS for monitoring of maximum and action levels for PCDD/Fs in feed and food matrices. As previously shown for other GC–MS/MS systems, the monitoring of two transition product ions provides selectivity for the analysis of PCDD/Fs, which is comparable to GC-HRMS at resolution 10'000. The proposed amendments of EU regulations for application of GC–MS/MS as confirmatory method can also be met by the APGC–MS/MS system. The application of faster clean-up methods shows, that the extraction and clean-up steps have considerable influence on the quality of the analytical results.

Keywords: *APGC–MS/MS, PCDD/F, PCB, food, feed*

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L-88
SIMULTANEOUS DETECTION OF RESIDUES FROM NINE ANTIBIOTICS FAMILIES IN MEAT SAMPLES USING A MICROSPHERE-BASED AFFINITY ASSAY

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Food-producing animals are treated with medicines to prevent or cure diseases. Residues of these substances in food products may threaten consumer's health. There is an urgent demand of effective screening tools for residues of veterinary drugs in livestock and in foods derived from these animals. For the detection of residues, different techniques are usually applied: microbial growth inhibition tests or ELISA immunoassays for screening, and liquid chromatography mass spectrometry (LC–MS) for confirmation purposes. These techniques have weaknesses in terms of sensitivity and specificity, or require expensive equipment and laborious sample preparation. Suspension array combined with flow cytometry is a new trend in immunoaffinity screening methods in food analysis. The use of microspheres enables the detection of multiple antibiotics families simultaneously in a few microliters of sample while still revealing the identity of each family. A multiplex microspheres-based flow cytometric affinity assay was developed for the simultaneous detection of amphenicols, fluoroquinolones, suflonamides, macrolides, lincosamides, pleuromeutilines, aminoglycosides, beta-lactams and polymixins residues in meat samples. Blank meat samples were spiked with antibiotic constituents from these antibiotic families, which are relevant in the veterinary field. Following two very simple and fast sample preparations, beads mix and specific binders are incubated with samples extracts. Residues could be detected below their respective maximum residue limits (MRLs) set in the Commission Regulation 37/2010. Validation of the assay according to the European Commission Decision 2002/657/CE will be the next step of the prototype development. The principle of flow cytometric immunoasssay method, sample preparation procedure, performance parameters of the method in comparison to other multi-analyte screening methods are presented and discussed. The method will be discussed, particularly, with focus on the increase of sample throughput and its potential to extend the range of analytes that can be determined simultaneously.

Keywords: *Antibiotic residues, flow cytometry, microsphere, immunoassay, screening.*

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L-89
THE USE OF ION MOBILITY ENABLED MASS SPECTROMETRY (IM–MS) FOR THE DEVELOPMENT AND CHARACTERISATION OF ROBUST ANALYTICAL METHODS FOR THE QUANTITATION OF VETERINARY DRUG RESIDUES IN FOODS OF ANIMAL ORIGIN

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Fluoroquinolone compounds are a class of antimicrobial agents which have been administered to livestock for (a) prevention and control of infections and (b) growth promotion. Due to concerns regarding the spread of resistant mircoorganisms in the human population the USA Food and Drug Administration introduced a ban on the use of enrofloxacin and ciprofloxacin in livestock production in September 2005. The use of antibiotic growth promoting agents in animal husbandry has been forbidden in the EU since 2006. Tandem quadrupole mass spectrometry is the technique most commonly employed for the quantitative analysis of veterinary drug residues, including fluoroquinolones, because it provides significant performance benefits in terms of selectivity and sensitivity. These benefits are attributed to the Multiple Reaction Monitoring (MRM) acquisition mode whereby two retention time matching transitions and ion intensity ratios are monitored in compliance with European Commission Decision (2002/657/EC)[1]. The selection of the most appropriate MRM transitions is critical and must be performed and validated in accordance with the 2002/657/EC. Many of the fluoroquinolone compounds are zwitterionic species, possessing multiple pKa values, thus careful method development and characterisation of both the extraction and analytical conditions is required to achieve accurate and reproducible results. In this study, we explore the use of Ion Mobility enabled Q–ToF–MS (IM–MS) as an important tool for method development to support the unequivocal identification and quantitation of incurred residues of fluoroquinolones in foods of animal origin. IM-MS uses a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility spectrometry (IMS) is a rapid, orthogonal, gas phase separation technique which allows another dimension of separation to be obtained within a Ultra Performance Liquid Chromatography (UPLC) timeframe. Compounds can be differentiated based on their size, shape and charge. In addition, both precursor ion and fragment ion information can be acquired in a single injection in a High Definition MS experiment; referred to as HDMSE. The unique insight provided by IM-MS has revealed characteristic ionisation patterns leading to the discovery of multiple sites of intra-molecular protonation within the fluoroquinolone class. Single component precursor ion MS and MSE fragmentation spectra were simultaneously generated for all ion mobility resolved components. These HDMSE observations may provide an explanation for the poor reproducibility and quantitation sometimes observed during the routine analysis of fluoroquinolone residues using triple quadrupole mass analysers monitoring specific MRM transitions. These observations may also be applicable to other classes of contaminants.

[1] Off. J. Eur. Commun. 2002; L221: 8-36. Commission Decision (2002/657/EEC)

Keywords: *Ion mobility, mass spectrometry, veterinary drug residues, fluoroquinolone, intra-molecular protonation*

Acknowledgement: *Monica Garcia-Lopez acknowledges the Spanish Ministry of Education, Culture and Sport for her postdoctoral contract.*

L-90
CRITICAL COMPARISON OF EU IDENTIFICATION CRITERIA VS EXPERIMENTAL METHOD PERFORMANCE: ARE CURRENT CRITERIA FOR LC–MS/MS FIT-FOR-PURPOSE?

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LC–MS/MS is one of the most widely used techniques for identification (and quantification) of residues. Although the same technique is applied, the parameters and criteria for identification vary depending on where in the world the analysis is performed and/or the purpose (e.g. pesticides, veterinary drugs, forensic toxicology, sports doping). The rational of these differences is not clear and in most cases the criteria are essentially based on expert opinions rather than underpinned by systematic experimental data. For pesticides in food/feed analyzed by LC–MS/MS in the EU, according to SANCO/12495/2011, identification is based on relative retention time and the ion ratio of ≥2 transitions, which need to fulfill certain criteria. These criteria originate from EU Commission Decision 657/2002. Since 2002, sample preparation became more generic, and LC–MS/MS matured and became a routine technique. However, the criteria remained the same. In the frame of the biannual revision of the SANCO guidance document, the deviations of retention time and ion ratios relative to reference values based on solvent standards have been systematically assessed for pesticides in a variety of fruits and vegetables. The study involved 120 pesticides (two transitions each) varying widely in polarity, sensitivity, and *m/z* of the transitions; 21 different matrices; blanks and spikes at 0.01, 0.05 and 0.20 mg/kg. The sample extracts and solvent standards were analyzed as one 120-injection sequence in five laboratories using different LC–MS/MS systems. The total data set consisted of responses observed in over 130,000 extracted ion chromatograms. The results clearly indicate that the current identification parameters and criteria are obsolete and need to be revised, and provide scientifically based input for new criteria. The results of this case study are believed to extend towards other application areas.

Keywords: *Analytical quality control, identification criteria, LC-MS, pesticides*

Acknowledgement: *Dutch Ministry of Economic Affairs; Monica Garcia-Lopez acknowledges the Spanish Ministry of Education, Culture and Sport for her postdoctoral contract.*

L-91
METALS AND METALLOIDS IN FOOD: SPECIATION AND -OMICS

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The consumer confidence in the quality and safety of food is a fundamental value of modern society. One of the parameters characterizing food both from the toxicological and nutritional point of view is the concentration of metals. The public perception distinguishes toxic elements (eg. Cd, Hg, or Pb) and essential nutrients (e.g. Fe, Zn, Cu or Se) but the scientific understanding of the risks and/or benefits associated with the presence of one or another metal is more complex. The same element can be essential or toxic as a function of concentration, speciation (the distribution among the different chemical compounds) and the presence or absence of other elements or potential ligands in a food. The rapidly developing industry of food supplements requires a complete account of all the forms of the supplemented elements present as only some of them have beneficial value while others may even be harmful. Adding to that that the trace element distribution and the isotope ratios of some elements (Pb, Fe, Sr) can be valuable tracers of food origin, there is a large number of situations where modern analytical tools for multielement food analysis, precise isotope ratios determination and speciation analysis become essential for the consumer protection. This contribution discusses the advances in analytical techniques addressing different aspects of trace elements in food. The democratization of inductively coupled plasma mass spectrometers (ICP MS) fitted with collision cells and the availability of reference materials has made total element analysis a quasi-routine task. ICP MS has also proved to be a convenient detector in gas and liquid chromatography rendering straightforward the analysis for particular species (methylmercury, inorganic arsenic, organotin, polybromine compounds). ICP MS – based coupled techniques have boosted exploratory speciation studies aimed at the identification of chemical forms (endogenous or artificially enriched) of trace metals and metalloids), their bioaccessibility and metabolism (e.g., selenomethionine, zinc and chromium species in functional food and food supplements) and the identification of metabolic pathways. Particular attention will be given to large-scale speciation approaches combining information obtained from the parallel use of elemental mass spectrometry (ICP MS) and molecular high accuracy Fourier Transform MS detection in chromatography and electrophoresis. The lecture will be illustrated with a number of examples addressing targeted and nontargeted speciation analysis. Analytical approaches aimed at the correlation of the multielement concentration pattern with genomic, proteomic and metabolomic data and their underpinned potential of the improvement of our understanding of the use of trace elements by plants will be will be highlighted.

Keywords: *Metals, speciation, metallomics, mass spectrometry, ICP MS*

L-92
METHYLMERCURY DETERMINED BY HPLC–ICPMS IN MARINE FOOD AND FEED; IN-HOUSE METHOD VALIDATION AND INTERLABORATORY COMPARISON

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Mercury (Hg) is one of the most hazardous compounds in our environment and is released through natural and anthropogenic processes. It occurs as elemental mercury (metallic), inorganic mercury or organic mercury. Inorganic mercury and the organic mercury compound, methylmercury, are the two major mercury species found in the environment. The toxicity is highly dependent on the chemical form and methylmercury is particularly toxic as it affects the functions of enzymes and is toxic to the nervous system (with the developing brain as most the most sensitive target). Currently only total mercury in foodstuffs is regulated by the European Union but accurate estimation of methylmercury exposure is needed for better evaluation of food safety. European Food Safety Authority established in 2012 a maximum tolerable weekly intake at 1.3 µg/kg body weight for methylmercury. Fully validated and standardized methods for determination of organic mercury levels in foods are missing. Here results from an in-house validation and an interlaboratory comparison study are presented for a simple HPLC–ICPMS method developed for methylmercury in marine based food and feed extracted with 5 M hydrochloric acid by sonication. The extraction step was carried out twice, the pH was increased and the extracts were diluted in mobile phase and filtrated (0.45 µm) prior to HPLC–ICP–MS analysis. Quantification of methylmercury (m/z 202) in the sample extracts was achieved by cation exchange separation (Hamilton PRP–X200, 150×2.1mm, 10 µm) and calibration standards prepared in mobile phase. The mobile phase (0.20 ml/min) consisted of 0.5% (w/v) L-cysteine, 50 mM pyridine, 0.8% (v/v) formic acid, 5% (v/v) MeOH and had a pH~3. The in-house validation included certified reference materials of marine origin (TORT-2, DORM-2 and DORM-3) and 4 other marine samples which were analysed in triplicates on 3 different days. The individual results were within the certified ranges. The limit of detection was 0.004 mg/kg and the in-house reproducibility standard deviations were less than ≤20% for samples containing 0.06 to 4.47 mg/kg. The small-scale collaborative study included 4 different laboratories that analysed 6 different marine food and feed samples in duplicate on 2 different days. For some samples one outlying dataset were excluded from the evaluation as Cochran or Grubbs outlier (following ISO 5725-2). The results were in general satisfactory in the tested range (0.2–5.7 mg/kg). The reproducibility standard deviations were less than ≤13%, HorRat values below 0.9 and the overall means for the reference materials (DORM-3, TORT-2, CE464 Tunafish) were in agreement with the certified values. Pitfalls for application of the method are; carry over between HPLC injections, calculations (exact extraction volume is critical for re-extraction) and careful interpretation of methylmercury certificates (which can be given as e.g. Hg, as CH_3Hg or CH_3HgCl).

Keywords: *Metylmercury, speciation, HPLC-ICPMS, validation, interlaboratory comparison*
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L-93
ANALYSIS OF MIGRATING COMPOUNDS FROM PLASTIC BABY BOTTLES WITH GC-QQQMS AND LC–QTOFMS: DEVELOPMENT AND APPLICATION OF A NON-TARGETTED EXTRACTION METHOD

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Bisphenol-A (BPA) is used as a monomer for polycarbonate polymers (water and infant feeding bottles) and epoxy resins (canned food packaging) from which it can be released into the food and so provide exposure of BPA to humans. Since BPA has endocrine disrupting properties, its use was recently prohibited for the production of polymers for food contact materials for children younger than 3 years old (European Commission, regulation (EU) N° 10/2011). Consequently, alternatives to polycarbonate food contact materials for children, such as polypropylene, polyethersulfone, polyamide, Tritan® or silicone baby bottles, have appeared on the market. Migration of BPA from polycarbonate has already been extensively studied. Unfortunately, the nature of (other) substances migrating from the polymeric alternatives to polycarbonate is much less known. The principal aim of our study was the identification of major compounds migrating from baby bottles. In this context, the use of simulants is prescribed in the legislation to mimic the testing of real foods. Specifically, we selected a mixture of water-EtOH (50:50) and a 3% acetic acid solution as stimulants for milk and juices, respectively. After sterilization of the bottle during ten minutes with boiling water, three migrations were performed during 2h at 7 °C. Firstly, the liquid-liquid extraction (LLE) from the simulants was optimized with a mixture of common organic compounds. To develop a robust and general method, a mixture of 17 chemicals covering a wide variety in polarity and chemical functionality was chosen to evaluate the extraction efficiency of n-hexane, iso-octane, ethyl acetate-n-hexane (1:1 and 1:3), MTBE and dichloromethane-n-hexane (1:1 and 1:3). The extracts resulting from the LLE step were analyzed on GC–QqQMS and LC-QTOFMS by monitoring specific mass transitions for each analyte and internal standard. The method performance was assessed by determining precision, accuracy, recovery, and matrix effects. Ethyl acetate-n-hexane (1:1) and dichloromethane-n-hexane (1:1) were the most efficient extraction solvents. Consequently, we opted for the non-chlorinated solvents and ethyl acetate-n-hexane (1:1) was selected for the application to real samples. Further, the possible release of unknown chemicals from polypropylene, polyethersulfone, polyamide, Tritan® or silicone baby bottles was assessed by performing a migration study with the simulants water–EtOH and 3% acetic acid. The migration solutions of the baby bottle samples were extracted and analysed on GC–QqQMS and LC–QTOFMS performing an untargeted database search using Wiley® and NIST® libraries. Various compounds such as alkanes, phthalates, amides, etc. were detected although the concentrations were relatively low. Additional toxicological research will be done in the future to evaluate the risk of these migrating compounds for human health.

Keywords: *Migrants, Baby bottles, Polycarbonate Alternatives, Liquid-Liquid extraction*

L-94
DIRECT ANALYSIS OF INTACT GLYCIDYL FATTY ACID ESTERS IN EDIBLE OILS USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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Glycidyl esters, fatty acid esters of glycidol, are by-products in edible oil processing. In the current contribution we present a new GC–MS based method measuring intact glycidyl esters. In comparison to the currently used LC–MS methods, the GC–MS equipment used in the new method is cheaper, easier to use and more abundantly present in oil and fat laboratories. The novel method includes a three step sample preparation in which the glycidyl esters are extracted from the edible oil matrix using acetonitrile, the co-extracted lipids are removed using a heptane wash and finally the GEs are isolated using NPLC. The isolated glycidyl esters are analysed as intact molecules using standard GC–MS equipment operated in the SIM mode. Short, well deactivated GC columns are used at a relatively high carrier gas velocity to avoid thermal degradation and artifact formation. Additionally, care was taken to obtain an optimal inertness of the GC injector. With these precautions a rugged method performance was obtained. The detection limits of the novel method are in the range of 0.01 mg/kg for the individual glycidyl esters. This corresponds to free glycidol levels of approximately 0.002–0.003 mg/kg. Recovery values ranged from 85 to 115% depending on the spiking level and the chain identity of the glydicyl esters. Additional trueness information was obtained by comparing our GC–MS data with those from LC-MS analyses. These comparisons indicate a good trueness. The repeatability and reproducibility of the method are better than 5–12% for individual glycidyl esters at levels above approximately 0.1 mg/kg. Over a period of 8 months of continuous use the method has been applied to all sorts of edible oils and oil blends, and was found to be very reliable and rugged.

Keywords: *Glydicyl esters, Glycidylesters, Glycidol, Edible oils, GC-MS*

L-95*
TRAVELING–WAVE ION MOBILITY PROVIDES ADDITIONAL CONFIDENCE IN THE ASSIGNMENT OF THE NATURAL AND RECOMBINANT FORMS OF BOVINE SOMATOTROPIN

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Under pressure due to the rising number of consumers and the competitiveness of food markets, some farmers may choose to improve their production using growth promoting factors, despite the potential risk for animal welfare and public health in terms of the chemical safety of animal products. In milk production, recombinant bovine growth hormone (rbGH) constitutes a very potent practice to increase milk yields up to 15%. Such a practice is authorized in some countries, but prohibited within the European Union since 1994. Analytical tools are therefore needed for the identification of rbGH-treated animals and the control of its potential misuse. The difference between the endogenous (bGH) and the most commonly used recombinant forms, is one amino acid located at the N-terminus of the protein, which modification generates a mass difference of ~60 Da. The close homology between recombinant and pituitary forms, as well as the low level of residues of bGH and rbG H in biological fluids, makes it difficult to discriminate rbGH-treated cows. Despite this, some efficient LC-MS strategies for blood have already been successfully implemented. Milk is currently considered as the most challenging matrix to detect rbGH in, because of both the complexity of the matrix which comprises a wide range of proteins exhibiting similar physicochemical properties to (r)bGH, and to the very low concentration expected (sub ppb level). So far, the developments of analytical strategies for the direct detection of rbGH in milk have not succeeded in detecting the hormone of interest in samples from treated animals. Therefore, even more specific and sensitive protocols are required to tackle this issue. Whenever high background noise is the cause of unsatisfactory sensitivity, an orthogonal separation mode, such as Ion Mobility Separation (IMS) may be of interest. With this technology, ions are separated based on the number of rolling back events they undergo, due to pulse height and gas pressure in Traveling-wave ion mobility (TWIM) cell. The rolling back events of a compound are closely related to its unique physiochemical properties including, conformation, size and charge. TWIM separation may be considered as an additional parameter for characterization the compound of interest. TWIM separation provides a 3D separation when coupled to UPLC and HRMS. Ionised species are differentiated according to their retention time, mass-to-charge ratio and mobility time which is related to their spatial arrangement expressed as measured Collision Cross Section (CCS). The CCS could be used as a new identification point for analyte confirmation purposes and provide additional confidence in the assignment of bGH and rbGH species. In addition, the use of IMS provides an orthogonal mode of separation to chromatography and mass resolution which enables clean-up of spectral information for better both specificity and sensitivity of the detection.

Keywords: *Somatotropin, recombinant growth hormones, supercritical fluid chromatography, traveling-wave ion mobility*

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L-96
TOWARDS LABEL-FREE QUANTITATIVE ANALYSIS OF PEPTIDES IN PROTEIN HYDROLYSATES

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Protein hydrolysates can contain a large number of different peptides. Even in a simple system (e.g. β-lactoglobulin hydrolysed by trypsin) more than 150 different peptides can be formed. To characterize hydrolysates in more detail than the normally used 'degree of hydrolysis', preferably one would like to have the absolute molar concentration of each peptide present in the hydrolysate. For absolute quantification of small numbers of known, specific peptides, isotope labelling has been used. However, for complex hydrolysates, with large numbers of peptides such an approach may quickly become impractical. In this study we show the use of reversed phase liquid chromatography followed by mass spectrometry as a label-free method for absolute quantification of large numbers of peptides. The absolute molar concentration of each peptide was calculated from the UV peak areas (at 214 nm) in the chromatogram. Molar extinction coefficients at 214 nm were calculated from the primary sequence. To account for the geometry of the detector a cell constant (K_{cell}) was introduced. The calculation was tested and validated for different standard peptides and two intact proteins (β-lactoglobulin and β-casein) using different elution conditions (e.g. flow-rate) and data collection conditions. Using the K_{cell} value determined from these experiments, the concentrations calculated from the UV peak areas are correct within 10 %, which is typically in the order of the experimental error. The benefit of the method is that the obtained concentrations are absolute, thus removing the need to use internal standards or other approximations. To verify the completeness of the analysis, methods were developed to improve sequence coverage analysis. Using a combination of the LC-MS methods and subsequent data analysis, the peptides in a hydrolysate of β-lactoglobulin were analysed. In these hydrolysates 45 peptides were annotated and quantified, resulting in a traditional sequence coverage of 100% and a modified, more accurate, sequence coverage (i.e. including all annotated peptides as related to all peptides which should theoretically be present given the identified cleavage sites) of >80 %. A quantitative analysis of the concentration of all the amino acids (present in the different peptides) showed a recovery of >80% as compared to the theoretical concentration based on the initial protein concentration.

Keywords: *Peptides, quantification, UPLC-MS, hydrolysates*

L-97
THE EVOLUTION OF ALLERGEN METHODS – WILL TOMORROWS METHOD JUST BE BETTER OR ALSO FASTER?

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Allergens regulation is by now implemented and enforced in most jurisdictions of the world. But does industry and enforcement have reliable methods for allergen detection, and therefore a sound basis for decision making? The presentation will show where current methods fail and why. The authors will demonstrate the link between a number of issues and the lack of appropriate validation and/or availability of reference materials. Cost/reliability/time issues will be addressed. In the last part of the presentation, an outlook will be given where the latest European Commission research will allow to close the gaps by linking clinical cohort study results to food manufacture procedures and to analytical results using mass spectrometry (LC–MS/MS), which will, for the first time, allow to establish a direct relation between allergic reaction and analytical result. This research is aimed to ultimately allow industry to make sound risk management decision and thereby protect their allergic customers.

Keywords: *Allergens, validation, pitfalls, LC–MS/MS, massspectrometry, European Comisssion*

L-98
MULTI-ALLERGEN DETECTION IN THERMALLY–PROCESSED BAKED GOODS BY MASS SPECTROMETRY

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Food allergy is a major public health concern that affects up to 8% of children and up to 2% of the adult population in the U.S. To protect the food allergic consumer with accurate food allergen labeling, reliable analytical methods are required for accurate allergen detection and quantification. These methods must be effective in spite of food processing-induced changes in the biophysical and immunological properties of multiple allergen proteins in a complex food matrix. In this work, a comprehensive liquid chromatography (LC)-mass spectrometry (MS) methodology is applied for simultaneous multi-allergen characterization and detection in thermally-processed foods incurred with milk, egg, and peanut. Combining enhanced protein extraction methodologies with high performance MS enables a peptide-specific view of changes in allergen proteins in foods prepared under varying conditions. Compiled data from a global proteomics approach are utilized to identify differentially-abundant peptides resulting from thermally-induced protein modifications. Furthermore, a comparative LC–MS/MS approach provides a platform by which to screen multiple allergens in a wide variety of food matrices for thermally-resistant or stable allergen peptide biomarkers. Identified peptide targets are then paired with isotopically-labeled analogs in the development of a multiplexed quantitative MRM (multiple reaction monitoring) LC–MS/MS assay for simultaneous detection of milk, egg, and peanut allergens in baked goods. Quantitative MS results are compared to those obtained with commercially-available ELISA kits. A combined analytical approach incorporating global proteomic screening with quantitative MS analyses yields an advanced understanding of fundamental changes in allergen proteins induced by food processing chemistry, thereby improving the performance of detection methods for allergens in complex food systems.

Keywords: *Multi-Allergen Detection, Mass Spectrometry, Thermal Processing, Allergens*

L-99*
LYSOZYME DETECTION IN WINES USING AN APTAMER-BASED BIOSENSOR AND SPR DETECTION

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The control of malolactic fermentation is an important aspect of wine processing. A recent alternative for sulfur dioxide is the utilization of lysozyme as antimicrobial agent during wine fabrication. Although lysozyme is very effective in preventing the growth of lactic acid bacteria the utilization of this protein is limited because it can remain in wine and this could cause allergenic reactions in hypersensitized consumers. The interactions between lysozyme and the phenolic compounds from wine is another drawback for its application [1]. There are a variety of analytical methods developed for the determination of lysozyme in wine based on chromatographic or immunosensing techniques like ELISA. Currently, there are some differences between the results provided by these methods based on different ways to ensure specificity (separation on chromatographic columns verses the use of bio recognition elements such as antibodies). A label-free aptasensor for lysozyme based on Surface Plasmon Resonance (SPR) detection, characterized by a detection limit of 70 nM, has been recently developed by our team [1]. In this work we report the further development of this sensor as a new tool for the sensitive detection of lysozyme in wines. A main challenge when it comes to application of new sensors to real samples is related to interferences due to the complex sample matrix. We minimized non-specific binding by coating our sensor with a self-assembled monolayer of a thiol containing ethylene glycol groups. On the other hand, it was found that lysozyme interaction with wine components affects drastically its binding by the aptamer. Thus, the aptasensor could be used to study these interactions. We supported our SPR data with electrochemical and enzymatic activity determinations. Several sample pre-treatment strategies (dilution, acid treatment, dilution with sodium chloride) have been examined. Concentrated solutions of sodium chloride allow freeing the protein bound to matrix constituents (such as tannins) but cause important conformational changes of lysozyme and affect the affinity of the aptamer for its target. We developed a procedure for the simple and accurate detection of allergenic lysozyme in wines. Studies with Romanian wines spiked with lysozyme allowed to calculate a recovery factor of the protein of 60–90% (lower for red compared to white wines). Our results agree well with an HPLC method and with literature data.

[1] Int. J. Food Microbiol., 2011, 148(3), 184-90. 2. Analyst, 2013, 138 (12), 3530–3537.

Keywords: *Aptasensor, SPR, non-specific binding, wine, lysozyme*

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L-100
SCREENING OF MULTIPLE ALLERGENIC INGREDIENTS IN FOOD MATRICES BY HPLC–ESI–ION TRAP MS

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In EU a total of 14 food allergens are regulated by the present legislation requiring their mandatory labelling on the respective food products whenever intentionally added to foods. The problem of inadvertent contamination of foods by allergens mostly occurring along food processing chains appears still uncovered. In order to protect allergic consumers' health and with the aim to deliver even more sensitive methods capable of unequivocal confirmation of the presence of the offending allergens, mass spectrometry has expanded its application field also intended as a suitable tool for food allergens detection, quantification and confirmation. In addition, features provided by the latest technology of mass analyzers have allowed the development of multi-target and sensitive MS based methods including quantitative aspects provided that some requirements are met for the final protein identification. In this communication the development of a method based on Selective Reaction Monitoring Mass Spectrometry (SRM–MS) for the simultaneous determination of potentially allergenic ingredients in complex food matrices will be described. The method is capable of detecting during the same run traces of egg, milk and soy allergens in a cookie chosen as model food. As first step, the optimal buffer and extraction conditions were tested and applied for the extraction of proteins from spiked cookies. The protein extract was then partially purified, enriched, enzymatically digested and directly analysed by HPLC–ion trap mass spectrometry. Different acquisition modes were investigated and compared. In particular the data-dependent function proved to be a useful tool for scouting the best peptide markers along with the most intense fragment ions to be further implemented in the SRM method. After selection of the most suitable peptides, a proper SRM method was built by monitoring specific transitions for each peptide tracing for the corresponding allergenic protein. Calibration curves were built and final LODs obtained were in the range of few ppm for all allergens analysed.

Keywords: *Multi-allergen screening, mass spectrometry, ion trap MS, spiked cookies.*

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L-101
DEVELOPMENT AND VALIDATION OF A TRIPLEX REAL–TIME PCR ASSAY FOR THE SIMULTANEOUS DETECTION OF CELERY (*APIUM GRAVEOLENS*) AND THREE MUSTARD SPECIES (*SINAPIS ALBA*, *BRASSICA NIGRA* AND *BRASSICA JUNCEA*) IN FOOD

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Celery allergy and mustard allergy are rather prevalent in Europe. In Switzerland and France about 30-40% of all food allergic patients are sensitised to celery and in France 1-7% of the inhabitants suffer from mustard allergy. Allergic reactions can affect the skin, the respiratory system, the gastrointestinal tract and/or the cardiovascular system. Celery and mustard are common ingredients in meat products, sauces, marinades and convenience products. Three varieties of celery (*Apium graveolens*), celery roots, celery stalks and leaf celery, and three mustard species, white mustard (*Sinapis alba*), black mustard (*Brassica nigra*) and brown mustard (*Brassica juncea*), are most frequently used in food. Due to their allergenic potential, celery and mustard ingredients in food have to be labelled according to the Directive 2007/68/EC.
The present study deals with the development and validation of a triplex real-time PCR method for the simultaneous detection of celery and the three mustard species in food. The method was developed by combining three previously published singleplex assays for the detection of white mustard [1], black and brown mustard [2] and celery [3]. Primers and TaqMan probes target at the *Sinapis alba* mRNA for MADS D protein, the *Brassica nigra* partial RT gene for reverse transcriptase from gypsy-like retroelement and the *Apium graveolens* NADPH-dependent mannose-6-phosphate reductase mRNA. The triplex assay neither showed cross-reactivity with other *Brassicaceae* nor other *Apiaceae* species. Low cross-reactivity (difference in the Ct value ≥ 12 compared to the positive control) was observed with caraway, cumin, fenugreek and ginger. The limit of detection (LOD) in serially diluted mixtures of DNA extracts from celery roots, white and black or brown mustard (1:1:1) was found to be 10 pg celery, 5 pg white, 0.5 pg black and 0.1 pg brown mustard DNA. The amplification efficiency was 101% for celery, 91% for white, 96% for black and 101% for brown mustard. In raw and brewed model sausages, the LOD was found to be 50 ppm celery, 50 ppm white, 1 ppm black and 1 ppm brown mustard. The triplex assay was applied to verify correct labelling of commercially available foodstuffs.

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Keywords: *Celery, white mustard, black mustard, brown mustard, triplex real-time PCR*

L-102A*
QUANTITATION OF GLUTEN IN WHEAT STARCH BY GEL PERMEATION CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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Celiac disease is an autoimmune, inflammatory disease of the small intestine triggered by the storage proteins (gluten) of wheat, rye, and barley. The only known effective therapy is a lifelong gluten-free diet and to ensure the safety of gluten-free foods, a threshold of 20 mg gluten/kg has been established by the Codex Alimentarius. Current methods for gluten analysis are PCR, mass spectrometry of gluten peptides and immunochemical methods (ELISA) based on antibodies against specific amino acid sequences from certain prolamin types. Assuming a prolamin/glutelin ratio of 1, the gluten content is calculated by multiplying the quantitated prolamin content by 2. Due to its textural properties, wheat starch is a common ingredient for gluten-free foods. While it is mostly well-accepted in Europe, its safety for celiacs remains controversial, especially in the US. During starch production partially water-soluble prolamins may be removed causing variable prolamin/glutelin ratios, which lead to an underestimation of the real gluten content. Therefore, the aim was to develop a new method for the quantitation of prolamin and glutelin in wheat starch by gel permeation chromatography with fluorescence detection (GP-HPLC-FLD). Detection of protein-autofluorescence at excitation/emission wavelengths of 277/345 nm offered a 110-fold increase in sensitivity for gluten solutions compared to UV detection at 210 nm. 22 food-grade and technical wheat starch samples (6 declared as gluten-free and 16 without specification of gluten content) were analyzed. One sample of gluten-free wheat starch, which was confirmed to be gluten-free by ELISA, was spiked with wheat flour to obtain defined amounts of gliadin and glutenin for matrix-calibration. 1 g of each sample was pre-extracted twice with salt solution to remove possibly interfering substances. Then the gliadins were extracted with 60% aqueous ethanol at 22 °C and the glutenins with phosphate-buffer (pH 7.6)/2-propanol (1+1, v/v) containing 5 mg dithiothreitol/mL at 60 °C. In a second batch, total gluten was extracted by omitting the step with 60% aqueous ethanol. The gliadin content of all starches was also quantitated by competitive and Sandwich-R5-ELISA. Except for two samples with very high gliadin contents, the results of gliadin quantitation by GP-HPLC-FLD and both ELISA methods showed a good agreement, but the Sandwich-ELISA tended to give lower values. Most remarkably, considerable amounts of glutenin (> 20 mg/kg) were detected by GP-HPLC-FLD in 9 out of 16 not specified starches and even in 4 out of 6 gluten-free wheat starches. Furthermore, the prolamin/glutelin ratios were highly variable and below 1 in 8 out of 22 samples. Multiplying the gliadin content by 2 thus leads to a clear underestimation of the real gluten content. Taken together, these results demonstrate the need for non-immunochemical methods capable of detecting both prolamins and glutenins to guarantee the safety of gluten-free foods.

Keywords: celiac disease, gluten, gel permeation chromatography, fluorescence detection

L-102B*
TOWARDS A MULTI COMPONENT INCURRED REFERENCE MATERIAL FOR FOOD ALLERGEN ANALYSIS

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Food induced hypersensitivity reactions (allergy, intolerance) affect an increasing ratio of the population. The effective treatment of these illnesses is the avoidance of the triggering proteins in the diets. For detection and quantification of these components, reliable, validated analytical methods are necessary. Several methods (ELISA, immunoblotting, PCR, MS techniques) are available for routine work. Among these the most commonly used methodology is ELISA. However, reference method has not been assigned yet. The lack of incurred reference materials (RM) also complicates the fulfilling of the method validation process and the determination of the accuracy of analytical methods. Today, a well identifiable research direction is the development of methods suitable for multi component analysis. In consequence of this R+D efforts, the development of multi-component RM is more than reasonable. In the past few years, our working group developed a complex model food matrix which is suitable for producing incurred reference materials. This processed food matrix (cookie) was applied for the evaluation of incurred RM candidates containing gliadin in defined amount and in homogeneous distribution. The characterisation of this RM was performed; the effects of food processing and the interactions with other food components were investigated with the help of this processed food matrix. After the successful completion of RM development, we turned towards the production and evaluation of multi-component RM matrices. On the basis of the relative incidence of hypersensitivity reactions, four components -gluten, milk, egg and soy- were chosen for our preliminary work. Our results show that the developed RM process is suitable for producing homogeneous distribution in case of all four allergens. The effects of food processing were also investigated, and a decreasing tendency in measured protein concentration was observed in all cases. The allergenic protein content was also determined in mono- and multi-component matrices, for identifying the effect of potential cross-reactions. In some cases, discrepancies can be observed which means that the cross reactions could not be excluded. For understanding the background of the observed phenomena, further investigations are necessary.

Keywords: *Food allergens, multi component reference material*

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ALLERGENS

(A-1 – A-20)

A-1
QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF CHOSEN LOW MOLECULAR WEIGHT ALLERGENS ISOLATED FROM CEREAL FLOUR AND CEREAL FOOD STUFFS

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Within last years, the occurrence of food allergens and corresponding food allergies has been increasing. Therefore, understanding and study of structure of individual allergen is required. Cereals contain proteins associated with both intolerances (e.g. coeliac disease) and allergies (e.g. bakers' asthma) [1]. The most widespread allergens include several groups of plant proteins belonging to prolamins, 2S albumin seed storage proteins, the nonspecific lipid transfer proteins, and the group of cereal α-amylase and protease inhibitors [2]. In this work, we focused on characterization of cereal glycoproteins because most of food protein allergens are glycosylated. As the cereal samples, barley (Hordeum vulgare) and wheat (Triticum sp.) were selected, because both of them represents one of the most important cereals with wide range of utilization in food industry. The attention was concentrated to water soluble fraction of N-glycoproteins in barley and wheat flour and two types of corresponding final cereal food stuffs (barley malt and wheat couscous). For glycoproteins capture and purification, we used various separation procedures including application of different types of HPLC (reverse phase, affinity) and gel electrophoresis. Isolated glycoproteins were subsequently analyzed by MALDI–TOF mass spectrometry. Several groups of glycoproteins, predominantly belonging to alpha amylase/trypsin inhibitors family, were identified. Some of them are even proven allergens according to available databases. Nevertheless, neither glycosylation nor function of most of other detected proteins (especially with higher molecular weight) has been described yet. Low molecular weight glycoproteins were furthermore subjected to more detail quantitative analysis. The representation of selected allergenic glycoproteins, confirmed in individual cereal samples and their corresponding food end-products, was compared and their relative quantification was performed using Isobaric tags for relative and absolute quantitation (iTRAQ).

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Keywords: *Barley, wheat, proteomics, glycoprotein, allergen*

Acknowledgement: *This work was supported by the Czech Science Foundation (grant No. P503/12/P395) and by institutional support RVO 68081715 of Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, v.v.i.*

A-2
IMPROVED DETERMINATION OF ALLERGENIC FRAGRANCES IN DETERGENTS AND PERSONAL CARE PRODUCTS IN MULTIPLE REACTION MONITORING GC–MS

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Sensitization, intolerance and (pseudo)allergenic reactions represent contrary answers to perfume and fragrances. According to EU Directive 76/768/EC a number of allergens should be labeled if they exceed a certain concentration. The concerning allergens are mentioned in Annex III of Regulation (EC) No 1223/2009. Regarding the detergents directive (EC) no. 648/2004 allergens should be labelled as stated in the cosmetic regulation from 0.01% (*w/w*). In this study suitable transition have been studied to identify >20 compounds more accurate by multiple reaction monitoring.

Keywords: *Allergens, detection, fragrance, GC, Triple Quad*

A-3
THE OAT MYSTERY – ARE THEY GLUTEN-FREE?

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There is an on-going debate whether oats can be tolerated by celiacs. On the one hand there is the issue that oats are often produced on shared equipment with wheat, rye and barley and therefore contaminated with these gluten containing grains. If the production of pure oats not contaminated with other gluten containing cereals is secured there are on the other hand still studies claiming that certain celiacs cannot tolerate pure oats. The structure of prolamins from oats differs from other gluten containing cereals. Latest research has shown a difference between oat varieties as well as differences in detection of gluten content. In vitro studies showed correlation of the reactivity of the monoclonal G12 antibody with the immunogenicity of prolamin extracts from different oat varieties. During the validation of Gluten G12 ELISA Test Kit about 80 different pure oat varieties have been analysed to check for positive or negative response. The positive results appear to be a specific reaction of the antibody with the toxic fragment, rather than a non-specific response. Therefore, the G12 antibody may shed new light on this debate by recognizing oat varieties that trigger a response in celiac patients. Validation data on different oat varieties are presented and conclusions for the use of Gluten G12 ELISA Test Kits to evaluate celiac safe oats are drawn.

Keywords: *Gluten, oats, mAb G12*

A-4
A BREAKTHROUGH IN FOOD ALLERGEN TESTING – DEVELOPMENT OF A1 MINUTE EXTRACTION PROCEDURE COUPLED TO A FAST ELISA ASSAY

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Today around 2–3% of the adult population and 5-8% of children are affected by food allergies. That is an impropriate response of the immune system to certain contents of food and drinks – mostly proteins – that are mistakenly believed to be harmful. Since already very low amounts of allergen can cause allergic reactions, which may lead to anaphylactic shock in severe cases, allergic persons must strictly avoid the consumption of allergen containing food. With allergens being the largest single cause of global product recalls, food manufacturers are seeking for fast and reliable methods ensuring the correct labeling of their products and preventing product recalls. Meeting these requirements, a new fast allergen ELISA assay was developed and validated for 6 different allergens, namely: Almond, Casein, Egg, Hazelnut, Macadamia nut and Peanut. All six allergen ELISA test kits incorporate an extremely fast extraction procedure – using only extraction capsules containing a proprietary powdered buffer and hot water – of only 1 minute and short incubation times in the ELISA assay of only 10 minutes. Besides a low cross reactivity, the kits also showed a good performance in intra and inter-assay precision validation with variation being below 15% in almost every kit. The limit of detection (LOD) was calculated based on the mean blank value of 19 blank extractions plus three-fold standard deviation. All kits have LODs ranging in the low mg/kg level (Almond: 0.5 mg/kg; Casein: 0.2 mg/kg; Egg: 0.5 mg/kg; Hazelnut: 1 mg/kg; Macadamia nut: 1 mg/kg; Peanut: 0.5 mg/kg). Recoveries were ranging from 64–130% when kits were challenged to recover spiked allergens in difficult food matrices such as chocolate, milk drinks, cookies and ice cream. The kits also showed an equivalent performance in recovery when being compared to established ELISA assays from the market. The validation showed that these new developed allergen ELISA assays are not only capable providing results in an extreme fast way, but also yield accurate results that you can rely on, making them suitable tools for the detection of allergens in every kind of foodstuff.

Keywords: *Food Allergens, ELISA, extraction*

A-5
INFLUENCE OF CLEANING PRODUCTION LINES FOR THE PRESENCE OF PEANUTS IN THE FINAL PRODUCT WITHOUT PEANUTS

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Food allergens are proteins in food that cause abnormal immune responses. Peanut is one of most important food allergens and its unintended ingestion could cause severe allergic reactions. Sensitive specific methods with low ppm (mg/kg) range are necessary to check whether a food contains any amounts of peanut. To display the impact of well cleaned production line and to emphasize an influence of contamination, 20 samples were analyzed in three different time periods. The samples were snacks without peanut, taken from several points of production, after intensive cleaning and two mixtures of spices added in two different points of production. For a quantitative analysis of peanut was used Veratox for Peanut Allergen (sandwich enzyme-linked immunosorbent assay S-ELISA). The results were determined at Biorad 680 microplate reader and expressed as ppm of total peanut. First, 10 samples were tested: two snack products without spices (taken from drum and conveyor belt 1) had results under the limit of quantification

Keywords: *Allergen, peanut, production line, contamination, S-ELISA*

A-6
MASS SPECTROMETRY BASED ALLERGEN DETECTION: APPLICABILITY OF PUBLISHED PEPTIDE SEQUENCES FOR THE QUANTIFICATION OF DIFFERENT PEANUT VARIETIES AND PROCESSED PEANUTS USING TRIPLE QUADRUPOLE LC/MS

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The prevalence of food allergies is increasing. Because of lacking causative immunotherapies to cure food allergy, the avoidance of the allergenic food is currently the only means to avoid unwanted allergic reactions. Thus allergic individuals have to rely on the list of ingredients and on precautionary labeling. The compliance of allergen labeling and allergen status of products can only be verified by the use of accurate methods for allergen detection. From enzyme linked immunosorbent assays (ELISA) and polymerase chain reaction (PCR) it is well known and has been published that the detectability may be influenced by varieties and especially by processing. Meanwhile several mass spectrometry (MS) based detection methods for allergenic foods have been published in literature, however no information about the detectability, meaning the response to different varieties and the influence of food processing is available. Since the MS based detection relies on pe ptides from few or only one species specific protein(s), the protein expression has to be ideally constant in all varieties/commodities and processed forms of the target food of interest. Using the example of peanut, we investigated the applicability of published peptides suggested for peanut detection in different varieties/ commodities and processed peanuts using a triple quadrupole LC/MS System. Peanut samples were extracted according to published protocols in slight modifications. The diluted protein extracts were reduced, alkylated and digested overnight using trypsin. The acidified extracts were directly injected into the LC/MS/MS system. Six peptides were used for detection with five to seven MRM transitions per peptide. For all peptides differently charged precursors were considered and all potential transitions were optimized for the LC/MS detection using Agilent`s 6490 triple quadrupole MS. In the optimized experiments 35 MRM transitions were monitored across the expected elution times using Dynamic MRM acquisition. All samples were detected as peanut by all target peptides. However, there was a change in the relative response within investigated peanut varieties shifted maximum by a factor of approximately two. In contrast processing of peanuts changed the response much more. An industrial produced peanut paste made from roasted peanuts resulted in only 10% of the average signal obtained from unprocessed peanut varieties. Mass spectrometry based detection methods have been suggested to be less prone to reduced recoveries due to processing. Using the published peptides the recovery of processed peanuts seems to be similar to ELISA and PCR. Even if the selected peptides are chosen in a way that they are not modified or are not affected by the Maillard reaction (e.g. epsilon amino groups on lysines) a reduced recovery due to processing has to be considered.

Keywords: *Peanut, MS, MRM, processing, response to different varieties, detection methods*

A-7
MEASUREMENT OF SI TRACEABLE QUANTIFIED ALPHA S1 CASEIN IN COMMERCIAL CASEIN ELISAS – TOWARDS METROLOGICAL TRACEABILITY

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Food allergy is a serious and potentially life-threatening condition; it affects around 1-3% of the population and prevalence is increasing. Allergenic foods are subject to labelling requirements and must be carefully managed during food production to limit cross contamination. Clinical trials with some of the major food allergens have determined 'threshold levels' above which an allergic reaction will be caused in a defined proportion of the allergic population. Eventually it is likely that 'threshold levels' will be formalised in guidelines or in law and it will be imperative that there are easily accessible, quantitative, metrologically traceable methods available to the food industry to validate and verify their control procedures. The current and most widely available methodology for allergen detection is based on immunoassay, ELISA, however there are well known drawbacks to such methods. Metrological traceability, to the International System of Units (MTSI) [1,2], the property of an analytical result which allows measurements made under different conditions to be compared in a meaningful way, is in its infancy with regard to food allergens. MTSI would greatly facilitate the standardisation of current analytical techniques for food allergens but as yet to our knowledge only a model MS system for MTSI of the allergen lysozyme in wine has been elaborated [3,4]. The use of quantification of an allergenic protein (alpha s1 casein from milk) quantified to the SI as a means to traceably compare results from different ELISA kits has been investigated. Initial results demonstrated that all three of the commercial kits tested were able to recognise the alpha s1 casein protein both before and after heating at 180°C albeit with quantification that varied between kits. The quantified alpha s1 casein was also added to a model food matrix (biscuit dough) and the efficiency of extraction of the casein from the raw and baked dough was measured using each of the commercial kits. Recoveries achieved were poor and our work continues with efforts to suggest means to improve the traceability and recovery of immunological approaches.

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Keywords: *Allergens, casein, immunoassay, SI traceable, mass spectrometry*

A-8
BIACORE AND ELISA DETECTION OF MILK PROTEINS IN RED WINE AS HIDDEN ALLERGENS AFTER THE FINING PROCESS: A COMPARATIVE STUDY

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Wine has been suggested as a potential source of allergens of animal origin, as residues after the fining process. Animal-derived fining agents are used both in red and in white winemaking processes worldwide, particularly caseinates from cow milk, egg proteins and gelatine from fish and other animals [1]. ELISA is considered today as the "gold standard" in the direct detection of allergens in foods, and several protocols for the quantitative detection of milk proteins have been developed [2] or are commercially available. Despite the large and consolidate use of ELISA methods, some limits arise, like the lack of comparability when different methods are used in ring tests, or their applicability towards foods that underwent particular processing able to partially inactivate antigens epitopes. The Biacore technique, based on the SPR (Surface Plasmon Resonance) approach, is a useful microsensor-based method applied on proteins detection. Up to now, a few papers describe the application of Biacore to detect allergenic proteins [3], and no red wine application is reported. Aim of this work was to compare classical ELISA to the Biacore approach for the identification of the presence of caseins in clarified red wine. To study the clarification process, a commercially available fining agent based on caseinates was added to a sample of cloudy red wine at different levels (up to 1200 ppm). After centrifugation, caseins were measured in supernatants before and after microfiltration (1.2 and 5 µm). For the detection of residues of caseins in filtered and non-filtered wine, a commercially available sandwich microtiter plate ELISA for caseins (α, β and κ) was compared with a specific fast biosensor inhibition immunoassay for bovine κ-casein. The calibration curves in wine showed similar measurement ranges for both assays. Filtering through 1.2 or 5 µm pore size removed all caseins from the wine tested in this study, to levels that do not exhibit a risk for people allergic to milk products even when 1200 ppm of caseinate was used. Results obtained in this study were consistent using two totally different immunoassays and detection methods, however, the effect due to the low solubility of the employed fining agent in our wine samples needs to be further investigated.

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Keywords: *Milk allergens, red wine, Biacore, ELISA, fining agents*

A-9
LABEL-FREE QUANTITATIVE PROTEOMIC ASSESSMENT OF ALLERGENIC PROTEINS EXTRACTED FROM PEANUT USING MOBILITY ASSISTED DATA INDEPENDENT LC–MS

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One of the barriers to the effective use of challenges in food allergy diagnosis is the lack of controlled and properly blinded foods containing well characterised allergenic food ingredients. These ingredients need to be representative of foodstuffs as they are eaten, and have an allergen profile representative of the food type. Major peanut allergens are characterised and defined by standardised nomenclature (e.g. Ara h 1 to 13), with many having multiple known sequences. Close homology is common within the 2S albumins (prolamins, Ara h 2, 6 and 7) and cupin family allergens (Ara h 1 & 3). The use of mass spectrometry (MS) allows individual detection of the isoforms of these allergens, whereas traditional immunodiagnostic detection methods (e.g. ELISA) do not provide this level of information. A label-free approach has been applied to the analysis of peanut allergens by implementing ion mobility, data independent analysis mass spectrometry (IM-DIA-MS) providing both qualitative and quantitative information in a single experiment as a means of characterizing the allergenic proteins from peanut flour. The results of this study have shown over 300 proteins were identified from tryptic digests of peanut extracts, including the major protein families associated with peanut allergy identified with a sequence coverage of 70% or greater. The peanut based allergens appear to show abundance over a wide dynamic range. In addition, the potential MRM transitions relating to peanut allergens have been derived from the discovery data and are available for acquiring using a tandem quadrupole instrument.

Keywords: *Ion mobility, allergens, peanuts, proteins*

A-10
RIDASCREEN®FAST SOYA (R7102) SANDWICH ELISA TO DETECT TRACES OF SOYA IN NATIVE AS WELL AS IN PROCESSED FOOD

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Soya belongs to the so called “Big 8” allergens and following the food labeling directives of many different countries soya has to be labeled as ingredient if used in food. Parallel to soybean allergy is one of the more common food allergies, especially among children and babies. Allergic reactions to soy are typically mild, however, although rare, severe reactions like anaphylactic shocks can occur. These patients have to avoid the intake of food containing soy. Therefore, detection and then labeling of soy and its components in food by the manufacturer is mandatory. The main allergens in soy are Glycinin (Gly m 6) and ß-Conglycinin (Gly m 5). R-Biopharms sandwich ELISA RIDASCREEN®FAST Soya (R7102) detects these two proteins in native as well as in processed food. Commonly, food or its ingredients are heated during the production. Therefore, it is very important to detect these processed soy-proteins, because they can lead to allergic symptoms in predisposed patients. Compared to other test-kits on the market, the ELISA RIDASCREEN®FAST Soya (R7102) is suitable for the recognition of strong heated soy proteins in various foods. This unique property makes the RIDASCREEN®FAST Soya (R7102) to the most reliable test system for the detection of soy allergens in raw and processed food.

Keywords: *Allergen, Soya, Soy Food, ELISA*

A-11
ALLERGEN DETECTION IN WINE BY LC–MS/MS

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In wine production fining a wine eliminates any appearance of cloudiness by removing sediment. In this process fining agents, such as casein, are stirred into barrels of wine where they act as magnets by picking up the sediment in the wine and depositing it at the bottom of the wine barrel. Once the wine has been clarified, racking of the wine is done to separate the wine from the sediment residue.
In 2011 EFSA concluded that wines fined with casein/caseinate/milk products may trigger adverse reactions in susceptible individuals following a survey of wine where the detection of casein was reported in trace amounts [<2 mg/L (2 parts per million)] in two (out of 32) experimental wines without bentonite treatment and in three (out of 61) commercial wines with unknown treatment] [1]. This fact together with new European Union legislation (that states that wine after 30 June 2012 wine must disclose on the label if fining reagents such as casein / egg ovalbumin have been use in processing)[2] has driven the need for methods which are capable of detecting casein products in wine at low levels.
Here we present new data using microflow LC in combination with a LC–MS/MS method developed on an AB SCIEX QTRAP® 5500 system utilizing the *Scheduled* MRM™ algorithm which detects casein in wine at sub part per million levels. The method utilizes a simple digestion of the protein in situ in the wine followed by dilution and injection and has been designed to limit extensive sample preparation and perform all protein modification in the same Eppendorf tube. In the presentation we will discuss the benefits of MicroLC over higher flow rate separations.

[1] Scientific Opinion related to a notification from the International Organisation of Vine and Wine (OIV) on casein/caseinate/milk products to be used in the manufacture of wine as clarification processing aids pursuant to Article 6, paragraph 11 of Directive 2000/13/EC – for permanent exemption from labeling. EFSA Journal 2011, 9(10), 2384.
[2] COMMISSION REGULATION (EU) No 1266/2010 of 22 December 2010 amending Directive 2007/68/EC as regards labelling requirements for wines.

Keywords: *Allergens, wine production, MicroLC*

A-12
A PRELIMINARY STUDY ON THE OCCURENCE OF PYRROLIZIDINE ALKALOIDS IN ITALIAN HONEY

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In the last decade, the health and safety of honey has been taken into consideration as it can be contaminated by drugs used to protect bees against a variety of brood disease and/or by natural substances (like heavy metals, pyrrolizidine, grayanotoxins). The contamination of honey with pyrrolizidine alkaloids (PAs) has been reported as potential health risk, thus to evaluate and characterize PAs contamination in Italian honey is the aim of the study. An approach based on the identification of a few markers of the most representative genus of plants producing PAs was adopted as suggested by EFSA (2011). The markers were chosen, as those most frequently occuring in plants in Italian regions by consulting melissopalynology data reported from literature. Samples of polyflora honey were obtained randomly from the market or from beekeepers of the Veneto region, and lycopsamine, heliotrine, echimidine, senecionine, seneciphylline, retrorsine were measured using a liquid chromatography coupled with a mass spectrometry (LC-MS/MS). The sample preparation was performed dissolving 5 g of honey in H_2SO_4 (0.05 M) followed by an SPE extraction using STRATA XC columns (200 mg/6 mL) for cleanup. The LC separations were performed on a C18 Hypersil Gold column (100 × 2.1 mm, 1.9 µm, Thermo Fisher Scientific, CA, USA) with gradient elution of ultrapure water and methanol both containing 0.1% formic acid. Mass spectrometric identification was done using a LTQ XL ion trap (Thermo Fisher Scientific, CA, USA), equipped with a heated electrospray ionization probe, operating in positive ion mode. The identity of each PAs was confirmed by monitoring via MS/MS the ratios of the two prominent product ions, previously selected. The selectivity, linearity response, trueness, precision (repeatability and within-laboratory reproducibility), limit of detection, limit of quantification and recovery were determined for validation purposes using blank honey sa mples spiked with PAs standard solutions at 3 different levels (1, 5, 25 ng/g). For all the analytes linearity of calibration curves was observed in the range of 0.25–50 ng/g and regression coefficient (r^2) was always > 0.99; mean recovery ranged between 91.6% and 104.3%; limits of quantification was 0.25 ng/g for all PAs and the repeatability and reproducibility values were always below 8.5% and 16.6% respectively. The validated method was then applied to 70 commercially honey samples for the survey of PAs in Italian honey.

[1] Scientific opinion on pyrrolizidine alkaloids in food and feed". EFSA Journal 2011; 9 (11); 2406.

Keywords: *Pyrrolizidine alkaloids, Italian honey, LC-MS/MS, food safety*

Acknowledgement: *Project partially supported by University of Padua 60A08-9748/13 to FC*

A-13
DIFFERENTIATION OF SCOMBROIDS BY PCR-BASED DNA ANALYSIS OF THE CYTOCHROME B AND PARVALBUMIN GENE

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DNA-based seafood authenticity methods are predominantly performed by PCR analysis. Scombroidae fish family contains several species with high values of commercialization, which are *Thunnus obesus, T. albacares, T. maccoyii, T. tonggol, T. alalunga, Katsuwonus pelamis, Auxis spp.*, and *Scomberomorus spp.* among others. Furthermore, most of world Tuna's supplies are captured in western central Pacific Ocean. However, Indo-west Pacific Ocean part urgently needs more concern in effective tuna fisheries management. On the other hand, an appropriate method to differentiate between closely related Thunnus species is continuously questionable either by genetic or morphologic identification. An accurate method of Thunnus species differentiation will support sustainable fishery and trade of tunas. Our present studies comprise:
(1) fish species identification using mitochondrial cytochrome b gene;
(2) differentiation of Scombroid fish from Indo-west Pacific and Indian Ocean by exon-primed intron-crossing (EPIC) PCR of a parvalbumin gene intron;
(3) new information about applicability of SSCP and RFLP technique to discriminate Thunnus species.
Using cytochrome b gene as an identification marker we could determine a number of SNP characteristics and SSCP electropherogram results within Scombroid group. Species-specific EPIC primers were successfully constructed and amplified from the third and fourth intron of parvalbumin gene. Moreover, SSCP and RFLP electropherogram results from parvalbumin gene intron show reliable differentiation of closely related Thunnus species. Therefore, these results showed potentially reliable species identification methods for high-priced tuna's products as well as other Scombroid fish. Difficulties and limitation of Thunnus spp. reliable identification are discussed.

Keywords: *Parvalbumin gene, EPIC–PCR, cytochrome b gene, Thunnus, species identification.*

A-14
UPLC ANALYSIS OF BIOGENIC AMINES IN DIFFERENT CHEESE VARIETIES

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High concentrations of biogenic amines can be found due to microbial activity intrinsic to typical fermented foods such as wine, fermented meat and especially cheese. During cheese ripening, accumulated free amino acids may act as precursors for the conversion into biogenic amines mostly affected by bacterial decarboxylases of a contaminating microflora. Thus, biogenic amines in foods are of main concern in relation to food spoilage/hygiene and food safety aspects. Considering the toxicological implications of these amines, and the general interest in occurrence data for "risk assessment" of fermented foods, the objective of this study was to analyse the concentration of biogenic amines in various commercial cheese samples (n=151) representing most common cheese varieties. AccQ-Fluor derivatizing reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) was used to analyze primary and secondary biogenic amines by ultra-performance liquid chromatography (UPLC). In general, cumulative levels of biogenic amines varied to a great extent with exceptional samples having amounts up to 150–300 mg/100 g cheese (e.g., Tiroler Graukäse with 313 mg/100g or Tiroler Almkäse with 185 g/100 g), whereas only 5% of the analyzed cheeses showed total concentrations higher than 90 mg/100 g (median 5.7 mg/100 g). Regarding the most relevant biogenic amines, histamine was found in 79% of all samples, with maximum concentrations for Tiroler Almkäse (116 and 82 mg/100 g), but only 5% of the cheeses had a histamine level above 17 mg/100 g (median 0.9 mg/100 g). For tyramine (72% occurrence; 5% > 37 mg/100 g), highest values were found for Tiroler Graukäse (160 mg/100 g), Tiroler Almkäse, French raw milk cheese, Olmützer Quargel or Harzer cheese (each ~50 mg/100 g; median 1.0 mg/100 g). Putrescine was detected in 70% of the cheeses (up to 80 mg/100 g for some acid-curd cheeses; median 0.6 mg/100 g; 5% > 26 mg/100 g). Cadaverine was found in 47% of the samples (5% > 22 mg/100 g), with highest concentrations for Harzer cheese and Olmützer Quargel (126 and 75 mg/100g, median 0.2 mg/100 g). Tryptamine had the lowest occurrence (15%; 5% > 8 mg/100 g) and a median concentration of 0.3 mg/100 g. In conclusion, high (and toxicologically critical) levels of biogenic amines are definitely not associated with a certain type of cheese (as it is sometimes reported in former literature), but may vary depending on a large number of different incalculable factors (e.g., hygiene during the whole cheese production process, number and class of contaminants, degree of proteolysis in cheese, uncontrolled technological aspects). For all analyzed cheeses, both the individual and the total amounts varied greatly, making it virtually impossible/inadequate to pinpoint certain cheese types as more potent sources for intrinsically high biogenic amine levels. Thus, obligatory monitoring of biogenic amines should be considered to ensure high quality and safety of cheese products in future.

Keywords: *Biogenic amines, cheese varieties, UPLC, AQC derivatives*

A-15
ORGANISING A PROFICIENCY TEST FOR FOOD ALLERGENS

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In order to comply with allergen labelling legislation and to safeguard the production of allergen free food, food producers apply Food Safety Management Systems (FSMSs) dedicated for allergens. An essential part of FSMSs are the checks for the presence of allergens during production processes and in end-products. These allergen analyses might be done "in house" but most producers outsource these analyses to specialised service labs. These labs can demonstrate their performance in proficiency tests in which the results of the analyses are evaluated against independent criteria. For official control laboratories in food and feed it is also a legal requirement to take part in proficiency testing. However, not always a suitable proficiency test is available. At the moment, hardly any proficiency tests for food allergens are organised. Therefore, there is definitely a need for such proficiency tests. Organising a food allergen proficiency test is a major challenge as there are still many problems related to allergen analysis. For instance lack of certified reference materials and incomplete extraction from foods leads to differences in results between different analysis methods. This puts a challenge to organising and evaluating proficiency tests for allergens. In this presentation we will report the process of organising a proficiency test for food allergens and the difficulties encountered. It deals, amongst others, with the selection and making of standards and samples, the analysis of the samples by the participants using their own methods, evaluation of the reported results, and the performance assessment of the participants.

Keywords: *Proficiency test, food allergens*

A-16
DETECTION OF CELERY BY ISOTHERMAL AMPLIFICATION OF DNA

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Celery is a known food allergen and the allergenic effect does not appear to be reduced by cooking or by any other food processing procedures. In general, the celery root contains more allergens than the stalk. Until now no antibody based assays are available for the detection of celery as cross reactions with parsley and carrot impede the reliable determination of celery in food products. Two strategies are available for the identification of celery derived food ingredients: mass spectrometry methods can be applied to quantify the allergenic proteins or – more frequently – PCR assays are used to detect a DNA fragment highly specific for celery. Although both techniques are well established and highly accurate, they have one major drawback. For both approaches expensive equipment and highly skilled personnel is required to perform the respective analyses. During the past decade novel methods for the amplification and detection of specific DNA were developed. In contrast to the more elaborate PCR technique these assays do not rely on expensive thermal cyclers but can be performed in a conventional heating block. In contrast to the characteristic temperature profile of PCR the whole assay runs at a constant temperature of 65°C. Among these isothermal methods the loop mediated amplification of DNA (LAPM) is the most prominent strategy for DNA determination. We have developed the first isothermal approach for the detection of celery. The here described LAMP assay uses the mannitol dehydrogenase gene as a diagnostic target. This gene is sufficiently selective to discriminate celery from its close relatives parsley and carrot. Besides the high selectivity of this assay we could demonstrate the superior sensitivity of this LAMP approach. Less than ten genome copies are required to produce a positive signal. The here presented isothermal approach is significantly less complex than PCR but can compete in terms of selectivity, sensitivity and robustness with the well-established gold standard.

Keywords: *Celery, isothermal DNA amplification, food allergens, PCR*

A-17
TOWARDS A MULTI COMPONENT INCURRED REFERENCE MATERIAL FOR FOOD ALLERGEN ANALYSIS

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Food induced hypersensitivity reactions (allergy, intolerance) affect an increasing ratio of the population. The effective treatment of these illnesses is the avoidance of the triggering proteins in the diets. For detection and quantification of these components, reliable, validated analytical methods are necessary. Several methods (ELISA, immunoblotting, PCR, MS techniques) are available for routine work. Among these the most commonly used methodology is ELISA. However, reference method has not been assigned yet. The lack of incurred reference materials (RM) also complicates the fulfilling of the method validation process and the determination of the accuracy of analytical methods. Today, a well identifiable research direction is the development of methods suitable for multi component analysis. In consequence of this R+D efforts, the development of multi-component RM is more than reasonable. In the past few years, our working group developed a complex model food matrix which is suitable for producing incurred reference materials. This processed food matrix (cookie) was applied for the evaluation of incurred RM candidates containing gliadin in defined amount and in homogeneous distribution. The characterisation of this RM was performed; the effects of food processing and the interactions with other food components were investigated with the help of this processed food matrix. After the successful completion of RM development, we turned towards the production and evaluation of multi-component RM matrices. On the basis of the relative incidence of hypersensitivity reactions, four components -gluten, milk, egg and soy- were chosen for our preliminary work. Our results show that the developed RM process is suitable for producing homogeneous distribution in case of all four allergens. The effects of food processing were also investigated, and a decreasing tendency in measured protein concentration was observed in all cases. The allergenic protein content was also determined in mono- and multi-component matrices, for identifying the effect of potential cross-reactions. In some cases, discrepancies can be observed which means that the cross reactions could not be excluded. For understanding the background of the observed phenomena, further investigations are necessary.

Keywords: *Food allergens, multi component reference material*

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A-18
QUANTITATION OF GLUTEN IN WHEAT STARCH BY GEL PERMEATION CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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Celiac disease is an autoimmune, inflammatory disease of the small intestine triggered by the storage proteins (gluten) of wheat, rye, and barley. The only known effective therapy is a lifelong gluten-free diet and to ensure the safety of gluten-free foods, a threshold of 20 mg gluten/kg has been established by the Codex Alimentarius. Current methods for gluten analysis are PCR, mass spectrometry of gluten peptides and immunochemical methods (ELISA) based on antibodies against specific amino acid sequences from certain prolamin types. Assuming a prolamin/glutelin ratio of 1, the gluten content is calculated by multiplying the quantitated prolamin content by 2. Due to its textural properties, wheat starch is a common ingredient for gluten-free foods. While it is mostly well-accepted in Europe, its safety for celiacs remains controversial, especially in the US. During starch production partially water-soluble prolamins may be removed causing variable prolamin/glutelin ratios, which lead to an underestimation of the real gluten content. Therefore, the aim was to develop a new method for the quantitation of prolamin and glutelin in wheat starch by gel permeation chromatography with fluorescence detection (GP–HPLC–FLD). Detection of protein-autofluorescence at excitation/emission wavelengths of 277/345 nm offered a 110-fold increase in sensitivity for gluten solutions compared to UV detection at 210 nm. 22 food-grade and technical wheat starch samples (6 declared as gluten-free and 16 without specification of gluten content) were analyzed. One sample of gluten-free wheat starch, which was confirmed to be gluten-free by ELISA, was spiked with wheat flour to obtain defined amounts of gliadin and glutenin for matrix-calibration. 1 g of each sample was pre-extracted twice with salt solution to remove possibly interfering substances. Then the gliadins were extracted with 60% aqueous ethanol at 22 °C and the glutenins with phosphate-buffer (pH 7.6)/2-propanol (1+1, v/v) containing 5 mg dithiothreitol/mL at 60 °C. In a second batch, total gluten was extracted by omitting the step with 60% aqueous ethanol. The gliadin content of all starches was also quantitated by competitive and Sandwich-R5-ELISA. Except for two samples with very high gliadin contents, the results of gliadin quantitation by GP–HPLC–FLD and both ELISA methods showed a good agreement, but the Sandwich-ELISA tended to give lower values. Most remarkably, considerable amounts of glutenin (> 20 mg/kg) were detected by GP–HPLC—FLD in 9 out of 16 not specified starches and even in 4 out of 6 gluten-free wheat starches. Furthermore, the prolamin/glutelin ratios were highly variable and below 1 in 8 out of 22 samples. Multiplying the gliadin content by 2 thus leads to a clear underestimation of the real gluten content. Taken together, these results demonstrate the need for non-immunochemical methods capable of detecting both prolamins and glutenins to guarantee the safety of gluten-free foods.

Keywords: *Celiac disease, gluten, gel permeation chromatography, fluorescence detection*

A-19
MATRIX VALIDATION: DETECTION OF BARLEY GLUTEN CONTAMINATION IN GLUTEN-FREE BEER USING THE EZ GLUTEN AND ALLER-TEK GLUTEN ELISA

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To meet the need for the detection and quantitation of barley gluten in beer, screening and quantitative immunoassays were validated in a single laboratory for these purposes. Both assays have been validated as AOAC Performance Tested Methods, and this data serves as an additional matrix validation for each. Sample replicates were tested at each stage of beer production using multiple yeast strains and methods of protein removal. Quantitation was performed using barley-specific standards based on barley flour extracts, and immunoassay results were confirmed using LC/MS for barley-specific peptides. Both immunoassay methods were sensitive to 5 mg/L (ppm) barley gluten, and quantitation was linear from 5 to 80 mg/L. Recovery for the barley-spiked worts ranged from 81–128% in the quantitative ELISA, and the LOD was < 1 mg/L. Both methods were found to be fit for purpose as screening and confirmatory methods, respectively, for the detection of low levels of barley gluten in beer.

Keywords: *Gluten, beer, barley, Skerritt*

A-20
DEVELOPMENT OF A SURFACE PLASMON RESONANCE BASED BIOSENSOR FOR OVALBUMIN DETECTION IN WHITE WINES

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Food allergy is nowadays regarded as a problem of public-health relevance, the main concern being the unintentional exposure of allergic consumers to the offending ingredient through allergen-containing food. Even a little intake of allergen can trigger unpredictable, highly variable reactions, depending on the dose and the sensitivity of affected individual, thus compelling the allergic consumer to avoid allergen-containing food totally. Complementary to confirmatory techniques, e.g., liquid chromatography mass spectrometry (LC-MS), rapid diagnostic tools are increasingly being promoted for food companies to verify the efficiency of their management schemes for food safety. In this communication, the development of a method based on Surface Plasmon Resonance (SPR) for the detection of ovalbumin in white wines will be described. A direct assay was designed, based on the use of polyclonal anti-ovalbumin antibody as specific receptor. Ovalbumin standard solutions were analysed to investigate the antigen-antibody interaction and different parameters influencing the final response were carefully investigated (i.e. pH, ionic strength, and additional surfactant concentration). The fine tuning of these analysis conditions allowed a sensitive response for the assay achieving a limit of detection in the low ppm range. The assay was tested for the analysis of ovalbumin in white wines artificially contaminated with the standard protein at different concentration levels. A limit of detection in the low ppm range was attained with minimum sample pretreatment.

Keywords: *SPR, biosensor, allergen, ovalbumin, wine*

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AUTHENTICITY, TRACEABILITY, FRAUD

(B-1 – B-53)

B-1
STUDY OF STABLE ISOTOPE COMPOSITION OF POLISH APPLES

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The subject of the study was determination the isotopic correlations existing between separated components of apple fruits. In this work we were looking for the correlations between the $\delta^{13}C$ values for sugars and organic acids of polish apples. The chemical and instrumental methods of separation: water, sugars, organic acids and pulp from fruit were implemented. IRMS technique was used to measure isotopic composition of samples. The European Union Regulations clearly show the tendency for application of the isotopic methods for food authenticity control (wine, honey, juice). Method of isotope ratio mass spectrometry is very effective tool for distinguish the food products of various geographical origin. The basic problem for identification of the sample origin is the lack of databases of isotopic composition of components and information about the correlations of the data. The final results of our study for original samples of apple fruits will be presented and discussed.

Keywords: *Fruit, stable isotope, sugars, organic acids*

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B-2
CHARACTERIZATION OF STABLE CARBON, NITROGEN AND OXYGEN ISOTOPE COMPOSITIONS OF TOMATO PASTES

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Tomato (*Lycopersicon esculentum*) is the most popular vegetable in the world. There are more than 150 million tons of tomatoes produced per year (FAOSTAT Database 2011), which is more than any other vegetable. The tomato is consumed in various ways such as raw, salads, sauces, ketchup and drinks. Tomatoes contain many nutrients (e.g. lycopene) with qualities that are beneficial to health. Tomato production has been reported for 175 countries (FAOSTAT Database 2011). The top five leading tomato-producing countries are China, India, the United States, Turkey, and Egypt (FAOSTAT Database 2011). Thousands of cultivars have been selected for optimum growth in differing growing conditions. Recently, characterization of isotopic composition of food materials has been used to verify their authenticity. Generally, the isotopic compositions of plant materials reflect various factors such as isotopic compositions of source materials (e.g., CO_2, H_2O, NH_4, and NO_2) and their assimilation processes as well as growth environments. For example, the carbon isotopic composition ($\delta^{13}C$) of plant organic matter has also been correlated with the amount of precipitation. The nitrogen isotopic composition ($\delta^{15}N$) mainly depends on soil nutrition. The oxygen isotopic composition ($\delta^{18}O$) mainly reflects that of local groundwater such as precipitation and meltwater. In this study, we determined $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of tomato paste samples from various cultivated areas in Australia (AU), China (CN), Chili (CL), Spain (ES), Italy (IT), Japan (JP), Portugal (PT), Turkey (TR), and the United States (US) to characterize their growth environments. Japanese tomato paste samples have lowest $\delta^{13}C$ and $\delta^{18}O$ values, ranging from −27.1‰ to −27.5‰ (n=10) in $\delta^{13}C$ values and from +20.3‰ to +22.5‰ (n=10) in δ18O values. Chinese tomato paste samples are characterized by high $\delta^{13}C$ values, ranging from -25.6‰ to -24.8‰ (n=9). The $\delta^{13}C$ values of plants depend on fractionation during diffusion of CO_2 into the leaf and subsequent photosynthetic metabolism and water use efficiency, suggesting that the carbon isotope discrimination is associated with well-watered conditions. We suggest that the large amount of precipitation in Japan would decrease their $\delta^{13}C$ values. Australian (+29.5‰ to +35.9‰ (n=10), Spanish (+26.8‰ to +29.5‰ (n=7)) and Portugal (+28.1‰ to +31.8‰ (n=10)) tomato paste samples are higher $\delta^{18}O$ values than other area. In general, the oxygen isotopic composition of plant materials mainly reflects that of precipitation, which mainly depends on latitude and altitudes. In fact, based on the GNIP/ISOHIS databases from International Atomic Energy Agency (IAEA) monthly weighted average data on the oxygen composition of precipitation, the precipitation in Australia, Spain and Portugal have higher $\delta^{18}O$ values. Thus, stable isotope compositions of tomato paste samples would reflect their growth environments.

Keywords: *Tomato pastes, stable isotope analysis, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$*

B-3
AUTHENTICITY RESEARCH AND LABEL CONTROL ARE INEVITABLE PARTS OF PROPER FOOD AND FEED SAFETY

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Official controls are carried out to verify compliance with rules on food and feed safety and labelling (Regulation (EC) No 882/2004). Safety requirements in general consist of maximum (residue) levels for specified hazardous substances in specified matrices or products, e.g. residues of veterinary drugs (Regulation (EC) No 37/2010), contaminants in feed (Directive 2002/32/EC). Therefore, establishment of the authenticity and composition of a sample, based on proper method availability [1], form a major part of the fundament of food safety research. Authenticity research and label control, conform Regulation (EC) 767/2009 (feed), Directive 2000/13/EC (food) and Directive 2001/110/EC (honey) a.o., are necessary for enforcement of legal limits, and provide guidance for applying the proper detection methods. Consumer protection and information will be facilitated in this way. Three examples will be presented to illustrate this importance: illegal contamination of beef with horse meat, adulteration of marshmallow roots with radix belladonnae containing atropine, and artificially produced honey mixtures sold as manuka honey. Several methods have been involved in these research examples, such as DNA detection (PCR), chemical analysis, profiling (near infrared), and microscopy. Conclusions and recommendations

• A range of methods may contribute to the analysis of composition and authenticity, such as DNA analysis, microscopy and profiling methods. Attention is needed for proper method development and availability.
• Authenticity research and label control should have a principal position in the enforcement of food and feed safety.
• An information strategy to stakeholders and consumers can only successfully be applied with sufficient information on identity and authenticity.

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Keywords: *Authenticity, composition control, fraud, label control*

Acknowledgement: *Resources for the research were provided by the Dutch Ministry of Economic Affairs.*

B-4
RAPID DIFFERENTIATION BETWEEN NATURAL AND ARTIFICIAL VANILLA FLAVORINGS USING DSA/TOF WITH NO SAMPLE PREPARATION

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Vanilla is the second most expensive spice and is widely used as a flavoring ingredient in food, beverage, cosmetic, pharmaceutical and tobacco industries. Vanilla extract comes in 2 forms; natural & artificial. Pure vanilla extract is made by soaking vanilla beans in a solution of alcohol in water. Artificial vanilla extracts are prepared from chemical synthesis of vanillin and ethyl vanillin from cheaper starting materials. Due to quality, price concerns and economically motivated frauds, it is important to differentiate between natural and artificial forms of vanilla extracts. In this work, we used a new open ambient source, DSA (Direct Sampling Analysis) coupled with TOF to differentiate between natural and artificial extracts with no sample preparation. Five natural and five artificial or imitation vanilla extracts were purchased and analyzed using the Axion 2 DSA/TOF system with no sample preparation. 10 µl of each sample was pipetted directly onto the stainless mesh of the Axion DSA. Mass spectra were acquired in negative ion mode in a range of *m/z* 50–700 at an acquisition rate of 5 spectra/s. All 10 vanilla extracts were analyzed by DSA/TOF with no sample preparation and no chromatography. The mass spectra for one of the natural vanilla extracts and one of the artificial vanilla extracts showed the presence of vanillin in both extracts but 4-hydroxybenzaldehyde was present only in the natural vanilla extracts. Similar data was obtained for the other four natural and two of the artificial vanilla extracts. This data shows that 4-hydroxybenzaldehyde can be used as a marker to distinguish between natural and artificial vanilla extracts using DSA/TOF. The ingredient labels for the other 2 artificial vanilla extracts showed the presence of benzoic acid as a preservative. Benzoic acid has the same empirical formula as 4-hydroxybenzaldehyde and therefore it is observed, in the spectra of these extracts, at the same mass as 4-hydroxybenzaldehyde. In order to distinguish between the presence of benzoic acid and 4-hydroxybenzaldehyde in artificial vanilla extracts, fragment ions were generated using collision induced dissociation (CID) at the capillary exit. The mass spectrum of a 10 ppm standard of benzoic acid, under these conditions, showed the presence of an ion at *m/z* 77.0397 Da which corresponds to $[M\text{-}H\text{-}CO_2]$- ion. This ion was not present however in the corresponding spectrum for a 10 ppm standard of 4-hydroxybenzaldehyde. A mass spectrum of one of the 2 artificial vanilla extracts showed an ion at *m/z* 121.0368 Da and an ion at *m/z* 77.0397 Da which confirmed the presence of benzoic acid in them. Furthermore an ion at *m/z* 165.0557 Da indicates the presence of ethyl vanillin further confirming that these 2 samples are artificial vanilla extracts. All mass measurements showed good mass accuracy with an error of less than 5 ppm.

Keywords: *DSA, MS*

B-5
CHARACTERIZATION OF WHITE TEAS AND GREEN TEAS USING HS–SPME–GC/MS

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Next to water, tea (*Camellia sinensis L.*) is one of the most widely consumed beverages in the world and is cultivated in more than 30 countries. The growing seasons, geographical regions, processing, and fermentation methods create many varieties that contribute to each tea`s uniqueness, especially to the tea aroma. Consequently, the volatile compounds are an important criterion in the quality control of tea [1,2,3]. The volatile compounds in the green teas and the white teas from different countries were analyzed and compared with regard to their composition. The concentrations of the volatile compounds measured in the samples varied considerably.

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Keywords: *White tea, green tea, HS–SPME–GC/MS, volatiles*

B-6
LUMICHROME AS A MARKER SUBSTANCE FOR THE DIFFERENTIATION OF UNIFLORAL CORNFLOWER- AND POLYFLORAL CORNFLOWER MIXED HONEYS

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Unifloral honeys become more and more popular due to their characteristic flavor and aroma. For these special products the consumer accepts higher prices than for common polyfloral honeys. Therefore, quality assurance according to consumer protection and fair trade is extremely important. So far, the microscopic pollen analysis is the method of choice. However, this is limited to honeys from the same plant species with similar pollen or underrepresented pollen, which finally leads to a wrong determination of honey identification with high probability. So, the IHC (International Honey Commission) requires alternative methods for evaluating the botanical origin by clear and objective parameters. Exceptionally, secondary plant metabolites such as polyphenols were applied to prove the authenticity of unifloral honeys [1–4]. In this study, the clear differentiation from German cornflower honeys (Centaurea cyanus) compared to cornflower-lime-mixed honeys is presented. Often, the timely same nectar availability leads to a mixture of the two honey flows. The low disposability of cornflower pollen complicated the pollen analyses. Oelschlaegel et al. have already proposed lumichrome as marker for the fluorescent cornflower honey [5]. An UHPLC–PDA–MS/MS–method allowed for the quantification of this substance next to other compounds in a number of different honey types. Thereby, cornflower honeys contained even two- to threefold higher contents of lumichrome than the other honeys. Furthermore, a comparison of the DAD profiles additionally allowed for a differentiation of unifloral honeys.

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Keywords: *Honey, Cornflower honey, lumichrome, riboflavin, fluorescence*

B-7
DEVELOPMENT OF A SCREENING METHOD FOR THE DETECTION OF 23 ILLEGAL DYES IN FOOD AND SPICES

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Synthetic dyes have been widely used as colouring agents for many years to colour various materials such as waxes, plastics, oils, textiles and shoe/floor polishes. The synthetic dyes compared with natural dyes have higher stability and lower production costs. Due to their chemical structure many synthetic dyes may have adverse effects on health including allergic and asthmatic reactions, DNA damage and some are suspected carcinogens and mutagens. There have been a number of instances of spices or other food ingredients such as dried chilli, chilli products, curry powder and palm oil, being contaminated with very low levels of prohibited dyes. Therefore to protect the consumer, food products need to be monitored and tested regularly for assurance that they are free of illegal contaminants. Reliable methods are required for detection of low levels of these colorants. The following method is described as a screening method for the simultaneous detection of 23 of the most significant illegal dyes (Orange G, Naphthol Yellow, Auromine O, Congo Red, Acid Red 73, Orange II, Rhodamine B, Metanil Yellow, Fast Garnet GBC, Sudan Orange G, Para Red, Dimethyl Yellow, Toluidene Red, Sudan Red G, Sudan I, Bixin, Oil Orange SS, Sudan II, Sudan Black B, Sudan III, Sudan Red 7B, Sudan Red B, Sudan IV) in foods and spices by high performance liquid chromatography with UV detection. The dyes to target were chosen to reflect those most often implicated in food recalls. The dyes were extracted from food or spice samples using a mixed solvent of 90/10 acetonitrile/acetone at a temperature of 40°C. Sample extracts were filtered and analysed by reverse phase high performance liquid chromatography using a gradient elution system of acetonitrile and ammonium acetate buffer at pH 3.0 with UV detection at a fixed wavelength of 510nm and 430 nm. The procedure was tested for linearity of response, limit of detection and recovery. Due to a lack of reference materials the validation procedures were based on blank samples that had been spiked with standard dye solutions at a relevant level. Each of the matrixes (chilli powder, turmeric, fennel, paprika, curry and palm oil) were analysed in duplicate on three different days. The average recoveries were in the range of 60–110%. Matrix interferences were detected in turmeric extracts and it is suggested that alternative chromatographic conditions be used to confirm the quantity of any dyes detected in this matrix. This method has potential as a simple, reliable and rapid screening method for priority illegal dyes in a variety of foods and species.

Keywords: *Dyes, spices, sudan*

B-8
PEPTIDES NATURALLY GENERATED FROM UBIQUITIN-60S RIBOSOMAL PROTEIN AS POTENTIAL BIOMARKERS OF DRY-CURED HAM PROCESSING TIME

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Spanish dry-cured ham is a very popular high-quality product with characteristic organoleptical and nutritional properties. The processing of dry-cured ham is very long, lasting up to 24 months, and its final quality and economic value is mainly given by the time of curing. For this reason, the development of efficient methodologies to control the processing through the analysis of the final product is fundamental. During the dry-curing process, the proteolysis of myofibrillar and sarcoplasmic proteins is one of the most important biochemical reactions occurring that results in the accumulation of numerous small peptides and free amino acids, which contribute to the development of dry-cured ham characteristic flavour and texture. Ubiquitin is a small regulatory protein that is encoded in mammals by four different genes UBB, UBC, UBA52, and RPS27A. UBA52 and RPS27A genes code for a single copy of ubiquitin fused to the ribosomal proteins L40 and S27a, respectively. The UBB and UBC genes code for polyubiquitin precursor proteins. Ubiquitin protein exists either covalently attached to another protein, or free. When covalently bound, ubiquitin can signal for the degradation of the attached proteins via proteasome, affect their activity, and promote or prevent protein reactions. The study of the peptides generated during the intense proteolysis of dry-curing processes could be a good way to establish a control of the time of curing. However, the identification of naturally generated peptides has become only recently possible through the use of the latest generation proteomic technologies, and no studies based on the peptides generated during protein degradation at different times of the processing have been done. In this study, the proteomic characterisation of the degradation of ubiquitin-60S ribosomal protein through the identification of the peptides generated at different times of processing (2, 3.5, 5, 6.5, and 9 months) has been done. A total of 19 peptides have been identified by mass spectrometry for the first time, showing the role played by muscle enzymes in the generation of these peptides through the dry-curing process. The fact that some of the identified peptides have been detected from 2 to 3.5 months, or that have only been detected after 9 months of processing, would suggest the potential use of these peptides as biomarkers of the time of processing.

Keywords: *Peptides, proteomics, proteolysis, dry-cured ham, processing time*

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B-9
STUDY OF PHYSIOLOGICAL WATER CONTENT OF POULTRY REARED IN THE EU

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The aim of this study was to determine the physiological water content in chicken raised and slaughtered in the European Union (EU) in 2012 and to compare it to the results of a study conducted in 1993 to assess whether the limits for 'extraneous water' in European legislation need to be revised. Seven Member States were chosen for participation in this study who accounted for more than 70% of total EU poultry production according to the figures published for 2009. Information was collected on current poultry rearing practices in the EU with respect to the most common breeds, weight classes, gender balance, flock/batch sizes and typical processing volumes. Using this information, a sampling plan was formulated that proposed collecting forty eight birds from each of the top EU poultry producing Member States. The sampling plan covered the key variables of breed, weight (light and heavy birds), gender, flock and cuts. Samples were collected from these seven Member States under the supervision of at least one LGC representative. The collected samples were frozen in the slaughterhouses or at the NRL in the Member State that samples were being sampled in and sent by overnight courier to LGC for sample homogenisation. Each sample was homogenised in accordance with ANNEX VIII of Commission Regulation (EC) 543/2008. Homogenised samples were stored frozen in aliquots until required for analysis. A stratified sampling plan was devised to distribute the samples to the participating eight NRLs, so that they each analysed a carefully selected set of samples from all seven Member States from which samples were taken. Thus the analyses were performed by eight NRLs. An analytical protocol stating acceptance criteria for the data was circulated to each laboratory. The laboratories were required to analyse a reference material (ERM®-BB501a) with each batch of samples, which was supplied. The results were entered into a standard electronic reporting form and returned to LGC for statistical analysis. Although the statistical analysis showed differences between chicken samples collected from different poultry producing Member States and the gender of the birds, these differences were small compared to the spread of results observed, and hence were considered not to be of practical significance. The 2012 study has confirmed that younger birds do have slightly more water and slightly less protein when compared to the results from the 1993 study. Although the changes appear to be small, they are significant; an appreciable number of chicken breast and leg cuts on sale in the EU would be expected to fail the limits set in European legislation if they remain unchanged. This study provides strong evidence to support a decision to amend the limits in European legislation so that they reflect chicken reared in the EU in 2012.

Keywords: *Water, poultry, moisture*

B-10
INTER-LABORATORY VALIDATION OF AN IMPROVED METHOD FOR DETECTING PREVIOUSLY FROZEN POULTRYMEAT BY DETERMINATION OF HADH ACTIVITY

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Poultry is a perishable product which requires careful control of temperature during production and retail processing. European poultry marketing legislation requires that poultry be marketed either as fresh or as frozen (or quick-frozen) poultry. It is not permitted to market poultry which has been frozen and thawed as fresh poultry. A robust analytical method that is capable of distinguishing between fresh and previously frozen poultry is therefore required for official control. A method to detect whether poultry and other meats had been previously frozen was developed and validated by collaborative study in the UK in 1997. The method relies on measuring the β-hydroxyacyl-CoA-dehydrogenase (HADH) activity of intracellular juice obtained from prepared test samples. The ratio of the HADH activity of sub-samples tested before and after laboratory freezing is compared to a reference cut-off limit to determine whether the sample has previously been frozen. The cut-off limit calculated for chicken breast meat in the original method validation study was 0.9. Subsequently, however, this value was found to be too high to effectively distinguish between chilled and previously frozen poultry. Additional work was funded by the Foods Standards Agency in the UK with the aim of improving the method to achieve a more effective cut-off limit for chicken. This work was carried out at LGC and improvements were made to the method which was then applied to additional poultry samples. The method was validated at LGC and a lower cut-off limit of 0.5 for chicken was recommended. The improved method has now been validated by collaborative trial with 12 UK Official control laboratories and 12 European National Reference Laboratories for water in poultry. The collaborative trial was successful in validating an analytical method that is suitable for the detection of previously frozen chicken which can be used to enforce legislation relating to the marketing of chicken within the European Union. The poster will present an overview of the need for this procedure for official control of poultry marketing in the EU, and will give details of the analytical method and the data obtained from the collaborative trial.

Keywords: *HADH, poultry, frozen*

B-11
THE USE OF RAMAN SPECTROSCOPY FOR THE DETECTION OF CONTAMINATION AND TRACEABILITY COMMODITIES USED IN THE ANIMAL FEED SECTOR

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As part of the EU FP7 project, Quality and Safety of Feeds and Food for Europe (QSAFFE), there are assessments being made on various "fingerprinting" methodologies with regards to determining conformity and geographical origin of imported commodities. One such technique that is being evaluated is Raman Spectroscopy, which arises from the inelastic scattering of light and is a non-destructive technique that is becoming increasing popular as a research tool in the food supply chain. Within Work Package 1 of QSAFFE, Raman spectroscopy was used to determine the adulteration of feed oils. Waste oils, such as transformer oils/mineral oils, were of interest and it was possible that they could contain dioxins/polychlorinated biphenyls (PCBs). Oils used in the feed industry, such as soya oil and basic vegetable blend oil, were adulterated with up to 25% mineral oil/transformer oil, Raman spectra of the mixtures were collected and chemometric analysis applied to the samples. Qualitative and quantitative chemometric models will be presented. Within Work Package 2 of QSAFFE, the botanical and geographical origins of commodities were investigated. Distillers dried grains and soluble (DDGS), a co-product of ethanol biofuel and beverage production, were highlighted as an emerging feed ingredient. In a crisis related to this commodity, such as contamination or authenticity issues, it would be beneficial to trace the geographical origin of the DDGS. Raman spectra of DDGS are affected to a large degree by fluorescence which masks any of the Raman signals. However, to overcome this, the oil fraction of the DDGS was extracted using accelerated solvent extraction (ASE), Raman spectra were collected and chemometric analysis applied to the data and qualitative calibration models were constructed. These models will be presented.

Keywords: *Raman Spectroscopy, Feed Oils, Adulteration, Geographical Origin, QSAFFE*

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B-12
CLASSIFYING THE GEOGRAPHICAL ORIGIN OF DDGS BY NEAR INFRARED SPECTROSCOPY COMBINED WITH CHEMOMETRICS

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With the recent and dramatic increase in bioethanol production, DDGS has quickly become a global commodity playing an important role in the animal feed industry. In case of a crisis which may be related to DDGS contamination or adulteration, it is of extreme importance to know the geographical origin of the specific commodity immediately to prevent further spread of potentially harmful material. In this study, 84 corn DDGS samples from Jilin Province of China, Heilongjiang Province of China, USA and Czech Republic were collected. Different near infrared spectrometers combined with different chemometric packages were employed by two laboratories (CAU and QUB) to investigate the feasibility of classifying geographical origin of DDGS. Results have shown that within spectral data process of SNV and 1st derivative pretreatment, PCA–LDA model developed by CAU and OPLS–DA model developed by QUB could perfectly discriminate DDGS samples from different geographical origins. These very promising results encourage the development of larger scale efforts to produce datasets which can be used to verify the geographical origin of DDGS. Such efforts are required to provide stronger feed security measures on a global scale.

Keywords: *DDGS, classification, geographical origin, near infrared reflectance spectroscopy, chemometrics*

Acknowledgement: *The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement n° 265702 (QSAFFE).*

B-13
OPTIMIZATION OF A POST-ANALYSIS PROCESSING FOR THE INTERPRETATION OF SSR FINGERPRINTS: RICE TRACEABILITY AS CASE STUDY

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Molecular markers are useful tools for assaying genetic variation. Among all, SSR markers are particularly employed in plant genetics and breeding, due to attributes like hypervariability, multiallelic nature, codominant inheritance and reproducibility [1]. Despite their apparent ease of interpretation, attribution of polymorphism based on DNA polyacrilamide gel electrophoresis (DNA PAGE) separation is time consuming, and may lead to errors when products are few bases distant. Moreover, low concentrated amplicons can be not displayed, leading to a general loss of information. Capillary electrophoresis-based genetic analyzers allow the operator to overcome these flaws, but they have a dramatic impact on the cost of the analysis. Microcapillary electrophoresis based on Lab-on-a-chip® technology allows to perform quick runs, providing a more detailed and objective measure of the amplicon size, compared to DNA PAGE. Also, the loss of information is reduced, since even weak signals can be detected, and the system permits a semi-quantitative analysis of each amplification product [2]. Nevertheless, due to its reduced discriminating power and low resolution, Lab-on-a-chip® technology has been considered unfit to perform complex genetic analyses [3]. Aim of this work was to set up a new statistical approach able to elaborate raw data of amplicon sizes coming from the microelectrophoresis instrument software. Samples of genomic DNA isolated from different varieties of pigmented rice were used as template, in order to study their phylogenetic relationships. Instead of choosing the classical binary model based on identity versus difference, we employed a probabilistic model of identity, with the aim of generating allelic clusters for each marker in our population study. Such approach led to the generation of genetic distances, that were used to create a genetic distribution. Locally cultivated Italian rice varieties clustered separately from other foreigner cultivars. The probabilistic approach was compared to the classical DNA PAGE outcome using a sub-sample of individuals, obtaining comparable genetic distances. Also, the use of the probabilistic method of analysis improved the resolution of groups of samples known to be closely genetically related, compared to the binary method applied to raw data coming from the microelectrophoresis instrument software. Finally, we suggest this new probabilistic approach of post analysis data processing to be used not only in combination with microelectrophoresis on chip but also on data coming from the classical DNA PAGE separation.

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Keywords: *SSR, microcapillary electrophoresis on chip, data processing, rice*

B-14
VOLATILE COMPOUNDS FINGERPRINTING ANALYSIS USING A FAST–GC SYSTEM: BOTANICAL AND GEOGRAFICAL ORIGIN IDENTIFICATION OF HONEY

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Botanical and geographical origin of honey is a key factor etheir for its commercial value or for the product characteristic. Currently, the diagnostic methods to evaluate the compliance of a honey sample to a particular botanical and geographical origin are based on organoleptic, physico-chemical and melisso-palynological analyses. The fingerprinting technique is one of the most promising answers to the traceability of food products. In particular, the fingerprinting of the volatile fraction may represent an interesting approach to the problem as these compounds are closely associated with the organoleptic perception of a product and then with its uniqueness. In this study, we used two types of honey, acacia and multiflowers, that are the most popular and widespread honey in Italy. These products are present on almost all the italian territory and are also largely imported from EU and non-EU. The botanical and geographical origin of samples were previously confirmed by classic melisso-palynological analysis and by a new PCR method developed by CRA–GPG. The volatile compounds analyses were performed with a HERACLES II Electronic Nose (Alpha MOS, France). It is based on the technology of ultra-fast chromatography with two short columns of different polarities and two Flame Ionization Detectors (FID). The statistical data analyses were performed using the AlphaSoft software (Alpha MOS). We analyzed the head-space of 46 samples (32 acacia and 14 multiflowers honey) of different geographical origin. As regards the results, the volatile compounds map based on Principal Component Analysis (PCA) showed a clear discrimination of acacia and multiflowers samples. PCA analysis of multiflowers honey samples of different geographical origins (Italian and Eastern European countries) showed a good separation of the maps of the volatile component. On the contrary, PCA analysis on acacia samples shows that the volatile compound maps overlap. This can be explained as differences of honey samples derived of single species, are dominant over the differences produced by geographic origin. In fact, multiflowers honey is given by mix of several species of flowers and their combination is strongly linked to their geographical origin. In conclusion, the fingerprinting analysis of volatile compounds is very promising in providing analytical information on food traceability and authenticity, also in view of the low cost and speed of execution. Further studies are currently underway to increase the number and the type of samples, in order to create more robust statistical model.

Keywords: *Honey, volatile compounds, fingerprinting*

B-15
CLASSIFICATION OF NEW ZEALAND HONEY BY MASS SPECTROMETRY AND CHEMOMETRICS

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New Zealand honeys are known for their antibacterial and antimicrobial properties; some (e.g. manuka/kanuka) more than others (rata, clover, kamahi, etc). Some high quality honeys from New Zealand are even used therapeutically, increasing their value and hence the motivation for fraud. The price of honey is based on its quality judged by botanical origin. Botanical origin is traditionally identified by microscopic analysis (palynology), but this is tedious and can be inaccurate because of the mixture of similar pollen (e.g. manuka and kanuka pollen are indistinguishable by conventional microscopy). Therefore, more advanced approaches are necessary for the reliable characterization of the botanical source. The feasibility of ultra-performance liquid chromatography – quadrupole time of flight mass spectrometry (UPLC–QToF MS) coupled to multivariate data analysis (MVA) for the classification of New Zealand honey (rata, clover, kamahi, and manuka/kanuka) was explored. Honey samples were extracted with 1% formic acid in methanol/water (50:50, *v/v*), shaken, sonicated, and injected after filtration. Using an untargeted metabolomics approach, reversed-phase chromatography and QToF analysis; some characteristic markers were detected for each honey variety. Using chemometrics, Principal Component Analysis (PCA–X), Soft Independent Modelling of Class Analogy (SIMCA), and Orthogonal Partial Least Square Discriminate Analysis (OPLS–DA) it was possible to discriminate between New Zealand honey samples of different botanical origin. Some of the characteristic markers that make a significant contribution to classification were detected for each honey variety. Based on exact mass measurement, elemental composition prediction and mass fragment data analysis, the main markers were characterized and identified using mass spectral databases.

Keywords: *New Zealand Honey, Botanical Origin, Mass Spectrometry, Chemometrics*

B-16
AUTHENTICITY OF VINEGAR BY GAS CHROMATOGRAPHY–COMBUSTION- ISOTOPE RATIO MASS SPECTROMETRY (GC–C–IRMS): A SURVEY OF VINEGARS IN THE SINGAPORE MARKET

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Vinegar is a popular condiment used widely in Western and as well as Asian cuisines. It is the product of the double fermentation of sugars to ethanol and then on to acetic acid. Balsamic Vinegar which hails from Modena and the Reggio Emilia region in Italy as well as the Zhenjiang xiang cu from the Zhenjiang region in China are famous vinegars fermented from different sources – the former from white grape musk and the latter from rice, which is typical of vinegars produced in Asian countries such as Japan and Korea. The price of vinegar is determined by the type of raw materials used for the fermentation, geographical location and also in some cases the type of process that is used during the manufacture. Fraud can occur in the form of mislabeling of the source from which the vinegar is fermented or adulteration of expensive varieties of vinegar with cheaper sources of vinegar. There are also concerns over the use of non-biological sources of acetic acid, e.g. petroleum-derived products to produce vinegar. Many techniques have been discussed in literature, including the use of SNIF–NMR, HS–SPME–GC–TC/C-IRMS and IRMS bulk analysis. In this study, GC–C–IRMS is used in the determination of $\delta^{13}C$ stable isotopic ratios of vinegar samples that are purchased from supermarkets in Singapore. The objective is to gain an understanding of the characteristic signatures of vinegars fermented from different sources and to check if there are any possible vinegar fraud issues in the Singapore market.

Keywords: *Vinegar, Acetic Acid, d13C Stable Isotopic ratio, GC–C–IRMS*

B-17
A NEW APPROACH TO IDENTIFICATION OF GEOGRAPHICAL AND SPECIES-SPECIFIC ORIGIN OF MEAT AND ROE

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Safety and quality of food products are crucial aspects of food security. Sharp increase of trade volume and complicatedness of supply chain during the recent decades has led to a range of issues concerning identity and safety of food products. Expensive commodities become a subject of fraud such as adulteration and falsification. This creates a serious threat for consumer health due to the unidentified origin of counterfeit products. Our work was devoted to the identification of geographical origin of the test objects by the determination of isotope ratio of light elements by EA-IRMS (Elemental Analysis - Isotope Ratio Mass Spectrometry) (DELTA ADVANTAGE). Species-specific origin of the test objects was determined by near IR spectroscopy (IR spectrometer by Frontier FT–IR–NIR with a NIRA device was used). We tested 120 samples of meat and 200 samples or red roe of various species and geographic origin. Sample preparation procedure included homogenization, freeze-drying and defatting. Geographic origin of all meat and roe samples was identified by isotope ratio of light elements by PCA (principal component analysis). IR spectra were processed using SIMCA algorithm (Independent Modelling of Class Analogy). 90% species identification was achieved.

Keywords: *Identification, geographical origin, PCA*

B-18
WINE AUTHENTICITY, QUALITY AND SAFETY ASSESSMENT BY MEANS OF 1H-NMR SCREENING

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Frequent wine scandals in recent years have led to the need for a technology that can rapidly assess both quality and safety of wine. Early fraud detection as well as protection of high-value products is of high importance to protect the vitivinicultural field. A new solution performing wine analysis by means of proton NMR spectroscopy has been developed in a joint effort by Bruker BioSpin and Winespin-Analytics, an established German wine analysis company. The fast and fully automated analysis requires only limited sample preparation and delivers a multitude of quality and safety relevant parameters within a few minutes. Furthermore, the reproducibility and transferability of the method, based on the usage of standard operation procedures, guarantees identical results across different analysis laboratories. This reliable screening method is providing targeted and non-targeted multi-marker analyses:
– Quantification results for 56 analytes, with comparison to official reference values and to the concentration distribution of authentic samples
– Prediction of authenticity parameters such as grape variety, geographical origin and vintage
– Detection of unusual analyte concentrations, even for compounds that have not been previously identified.

Keywords: *Wine, authenticity, fraud, 1H-NMR*

B-19
DETECTION OF ADULTERATED COFFEE BY 1H-NMR AND CHEMOMETRICS METHODS

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The production of coffee in Colombia has decreased during the last decade, but its quality has maintained and even progressed. Colombia imports large quantities of coffee for its internal consumption as it obtains high prices for its coffee on the international market. This situation makes the implementation of methods able to detect frauds, i.e., adulterated coffees imperative. Here we present an expert system running in a fully automated manner and enabling to discriminate Colombian coffee beans from other countries beans, including neighbouring countries on the same continent. Since Colombian coffee is exclusively from Arabica specie, the problem can be recast into discriminating Colombian Arabica vs. other Arabica coffees. Our system has been designed for coffee extracted in non-deuterated solvents, thus ensuring lower operational costs, and consists in a cascade of binary partial least squares discriminant analysis (PLS–DA). The power of discrimination is very high as we report sensitivities and specificities ranging from 91% to 100% using a real data set of over 500 samples from 25 countries and 3 continents. Finally, upon detection of non 100% Arabica coffee, a PLS regression is used to quantify the ratio Arabica/Robusta. The results showed that quantification is possible with high accuracy.

Keywords: *Coffee, authenticity, 1H-NMR*

B-20
DNA-BASED ANALYSIS OF GENETIC DIVERSITY AND TRACEABILITY FOR IVORY SHELL (*BABYLONIA AREOLATA*)

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For promoter the production traceability of seafood products in Taiwan, we need to control quality effectively in identify the seafood species. Propose of this study is to analyze the gene diversity and identification of high values seafood, Ivory shell (Babylonia areolata), in Penghu Island by molecular marker technology and built the database. Total of 36 Ivory shell and other Babylonia samples from cultivation and wild were confirmed to the species and analyzed for inter simple sequence repeat (ISSR) method, mitochondrial DNAs, and SSCP (single-strand conformation polymorphism) method. The results were shown ISSR3, ISSR7, and ISSR13 primers of ISSR method and mitochondrial DNAs have good discriminate powders for inter-species and intra-species. Conclusion, ISSR, COI genes with SSCP method could detect different species and source seafood sample in time and get highly financial benefits.

Keywords: *Babylonia areolata, ISSR, mitochondrial DNAs, SSCP*

B-21
ADULTERATION IN FRUIT JUICES: A SOLUTION TO A COMMON PROBLEM WITH THE USE OF HIGH RESOLUTION LIQUID CHROMATOGRAPHY, UV DETECTION, QUADRUPOLE–TIME OF FLIGHT MS AND MULTIVARIATE DATA ANALYSIS

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Economically motivated adulteration of food has emerged as a growing problem in the food industry due to its extremely lucrative outcome. Economic adulteration has far reaching consequences in the food manufacturing chain, it impacts the profits of reliable food producers and can pose potential threats to the health of unsuspecting consumers. The process of adulteration includes unacceptable enhancements, dilution and/or substitution with less expensive ingredients, failure to declare contamination and inaccurate or misleading labeling of a product or ingredient. There is a great need for highly informative analytical testing methods to help to authenticate ingredients and finished products. In this study, pineapple juice samples were analysed by high resolution liquid chromatography coupled with photodiode array and accurate mass detection. Multivariate data analysis techniques including principal components analysis (PCA) and orthogonal partial least squares to latent structures data analysis (OPLS–DA) were applied to the data. Using specific information derived from the data analysis a database search allowed the identification of several citrus compounds in some of the commercially available juice samples claiming to be pure pineapple. The compound identities were confirmed using standard compounds.

Keywords: *Adulteration, authentication, juice, PCA, TOF*

B-22
DETECTION OF SHIIKUWASHA JUICE ADULTERATION BY CALAMONDIN JUICE USING VOLATILE COMPONENTS

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Objective Shiikuwasha (*Citrus depressa Hayata*) has been a very popular fruitlet as a flavor enhancer in Okinawa, Japan, and contains large amount of nobiletin reported to show anti-tumor activities [1] in the edible part of this citrus [2]. Therefore, market of Shiikuwasha products has grown rapidly by today's health-conscious consumers. As Shiikuwasha farmers could not keep up with the supply of this fruit, Shiikuwasha juice adulterated with Calamondin (Citrus madurensis LOUR.) juice produced in Taiwan and Philippines is widely commercialized. The objective of this work was to investigate the volatile compounds to detect simply the Shiikuwasha juice adulterated with Calamondin juice.
Methods Shiikuwasha juice, Calamondin juice and 10 commercial Shiikuwasha juice was used for the experiment. One milliliter of sample juice was placed in a vial. The vial solution was held at 40°C, and SPME fiber was introduced into the headspace of vial and kept for 20 minutes. Heating of GC column-oven was stopped and a column head was dipped into liquid nitrogen to collect the volatiles in splitless mode. Then, the SPME fiber was introduced into the injector of GC and kept there for 7 minutes with cryofocusing. Headspace gas was analyzed by GC and GC/MS.
Results Chromatograms with high resolution were obtained by HS–SPME–cryofocusing, and 39 aromatic components were identified or presumed. Calamondin contained a slight amount of gamma-terpinene (1.7%: composition rate) as compared with Shiikuwasha (17.3%), it is supposed gamma-terpinene detected Shiikuwasha juice adulterated with calamondin juice. We obtained γ-Terpinene ratio (gamma-terpinene peak area/total peak area). gamma-Terpinene ratio was with a 0.07–0.11 % range for 3 commercial juices, and these juices were doubt adulterations with calamondin juice.
Conclutions γgamma-Terpinene was a useful volatile marker component to detect Shiikuwasha juice adulterated with Calamondin juice.

[1] A Murakami et al. (2000) Cancer Res., 60, 5059–5066.
[2] S Kawaii et al. (1999) J. Agric. Food Chem., 47, 3565–3571.

Keywords: *Citrus volatiles, gamma-terpinene, adulteration, Shiikuwasaha, Calamondin*

B-23
DETECTION OF UNDECLARED ADDITION OF SYNTHETIC ACETIC ACID TO CANNED PRODUCTS USING SNIF–NMR AND IRMS

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The determination of synthetic acetic acid in vinegars and canned products is a specific interest in the food industry. Significant problem is the vinegar adulterated with diluted synthetic acetic acid. Prominent Czech producers of vinegar have repeatedly warned against suspected vinegar in the distribution chain. It can be expected to find canned products in the market, in which synthetic acetic acid diluted with water is intentionally used as the substitute for vinegar, while the addition of synthetic acetic acid is not indicated on the product label. For the detection of undeclared addition of synthetic acetic acid to canned products we applied isotopic methods, SNIF-NMR ($^{2}H/^{1}H$; site-specific natural isotopic fractionation-nuclear magnetic resonance) and IRMS ($^{13}C/^{12}C$; $^{18}O/^{16}O$; isotope ratio mass spectrometry), which both characterize the botanical origin of acetic acid and also detect adulteration of vinegar using synthetic acetic acid; they seem to be t he most reliable tools for authentication of vinegars in canned products. The aim of the study was the optimisation of SNIF–NMR and IRMS spectrometry methods for the atypical matrix and the determination of isotope ratios in a pickle of canned vegetables (traditional pickled cucumbers) to prove their adulteration or authenticity. We analyzed the following set of canned products: pickled cucumbers and sauerkraut with the declared content of vinegar purchased in the Czech market, model pickled cucumbers (pickle as mixture of spirit vinegar and synthetic acetic acid in different ratios) and model pickles prepared with different synthetic acetic acids diluted with water. Isotope analyses, coupled SNIF–NMR and IRMS methods, has been confirmed as a suitable tool for assessing the authenticity of canned products; they has enabled detection of synthetic acetic acid addition above 20% from total acidity.

Keywords: *Vinegar, canned products, synthetic acetic acid, SNIF–NMR, IRMS*

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B-24
EVALUATION OF HERB AND FRUIT JUICE ADULTERATION AND AUTHENTICITY BY COULOMETRIC ARRAY DETECTION AND PATTERN RECOGNITION ANALYSIS

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Although the adulteration of herb and fruit juice is a frequent phenomenon, there are few simple methods available for the screening of large numbers of commercial batches of product. The challenge arises from the complexity and variability of genuine material combined with unrelenting conduct of adulteration. Herb and fruit variety, growing region, season, ripeness, and processing methods all contribute to the variability of the authentic product, making unambiguous characterization difficult. Currently, one of the most reliable and applicable authentication methods is based on analytical chemical fingerprinting (untargeted metabolomic) techniques. Gradient HPLC with coulometric electrochemical array detection is particularly suitable for generating information rich metabolite fingerprints of endogenous electroactive metabolites termed the "redoxome". The analytes that form the redoxome reflect an organism's health or disease state, and in addition fo r herbs and juices, is comprised of compounds that influence color, flavor, nutritional value, stability and aroma. Such fingerprints can be interrogated using pattern recognition and unsupervised statistical programs such as principal-component analysis (PCA) to evaluate the authenticity or geographic origin of a given sample by comparing its chromatogram with a compiled population of authenticated reference samples in the database. In order to test the applicability of our technique, a generic gradient HPLC method with coulometric electrochemical array detection was developed that was capable of simultaneously measuring several hundred analytes in a single sample. Data were then interrogated using PCA to determine the minimal level of adulteration that could be detected. Three sample sets were chosen to test our approach. First, intentional blending of authentic oregano herb with typical adulterants; second, blending of pure orange juice with other fruit juices; and third, adulteration of orange juice with either peel or pulp wash.

Keywords: *Herbs, Fruit Juice, Adulteration, Authenticity, Coulometric Array Detection, Pattern Recognition Analysis*

B-25
DETERMINATIONS OF INORGANIC ANIONS AND ORGANIC ACIDS IN BEVERAGES USING SUPPRESSED CONDUCTIVITY AND CHARGE DETECTION

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Organic acid profiles in fruit juices are important in the beverage industry to characterize flavor, maintain product quality, and to meet labeling requirements. To analyze organic acids and anions of strong acids, such as chloride and sulfate, which are also present in beverages, ion chromatography with suppressed conductivity is the ideal analytical method. Unlike the anions of strong acids which are fully ionized, organic acids are weakly ionized and can exhibit lower conductivity responses versus concentration than the strongly ionized anions. The new Thermo Scientific Dionex QD Charge Detector promotes complete disassociation of many weakly disassociated compounds by drawing a current at a fixed potential. As a result, the charge responses of singly-charged organic acids and doubly-charged and triply-charged organic acids are proportionally higher than conductivity. Here we demonstrate separations of organic acids on a 4-µm particle size, capillary anion-exchange column. Four µm particle columns produce high efficiency separations but also have higher system backpressure, and therefore can only be used on a high-pressure capillary IC system. Capillary IC at µL/min flow rates is always on and ready for analysis and requires only 5.2 L/yr of deionized water. The results show comparably higher QD response for organic acids compared to chloride and sulfate. Additionally, use of CD/QD ratios to assess peak purity is demonstrated, thereby improving reporting accuracy.

Keywords: *Organic acids, juices, ion chromatography, suppressed conductivity, charged aerosol detection*

B-26
A NEW APPROACH TO SPECIES DIFFERENTIATION IN FOOD AND FEED

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The identification of animal species in meat and meat products is of great importance in the context of quality assurance for both the food and feed processing industries, particularly following the UK outbreak of BSE. Current methods are unable to achieve the necessary requirements, as they often cannot verify the presence of animal material in food and feed. Because numerous and sometimes exotic animal species are used in food and feed production, it is a great problem for the processing industry to examine the labeling of raw products. The aim of this study was to improve a detection method for animal species differentiation, based on the analysis of terminal restriction fragment length polymorphisms (T-RFLPs). We aim to improve the existing method by broadening the field of application (e.g. to include animal feed) and enhancing the sensitivity of detection. Furthermore, the existing animal-derived DNA data-base should be extended. The T-RFLP method for animal species differentiation, completely avoids the disadvantages of conventional methods. By introducing an intermediate step - a so-called nested PCR – it is possible to analyze not only food, but also feed – depending on the processing degree. Initial results indicate that the method can also be successfully used for the analysis of animal leather or skins. Moreover, this method could be used for forensic analysis, and to monitor the illegal trade of exotic and protected animals. This improved version of the detection system now allows all the animal species in a given sample to be detected simultaneously, considerably shortening the overall analysis time. The method is powerful enough to determine the species present in highly processed feed, within which the DNA tends to be extensively degraded, and to detect components which represent only 0.5% of the total. It is possible to distinguish between very closely related animal species. Even the presence of exotic anima l species such as crocodile, can be verified easily. Currently, the method allows the detection of more than 50 animal species. T-RFLP is a rapid and sensitive method for the identification of animal species which entirely avoids the disadvantages of conventional methods. Currently, there is no other method available which can identify all the animal species present in a sample in a single reaction. T-RFLP analysis has many applications, and contributes an important and efficient method for consumer protection.

Keywords: *Animal differenciation, T-RLFP, simultaneous detection*

B-27
POLYPHENOL ANALYSIS OF RED WINES USING LC–MS/MS

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Polyphenols contribute to the flavor, mouth-feel and color of a wine. However, aside from these aesthetic qualities, the polyphenol character of a wine indicates the region and variety of grapes used to produce the wine. The locality of the grapes used to produce a wine is significant with regards to labeling. In fact, most major wine making regions have stringent labeling regulation regarding the percentage and origin of grapes used to produce a wine. In extreme cases, cheaply produced wines are labeled fraudulently as being from a more highly-regarded wine region. Consequently, there is a lot of interest in monitoring polyphenols in wine for research, quality control and fraudulent investigation. The most sensitive way to monitor polyphenols is using liquid chromatography coupled with a mass-spectrometer. The presented method utilizes a simple dilute-and-shoot sample preparation procedure, followed by LC–MS/MS analysis in the negative mode. Several different wine varietals from different wine making regions were analyzed. In each wine the observed polyphenolic compounds were quantified against a calibration curve. The quantitative results were then used to compare the polyphenolic profile of each wine with respect to its variety and location.

Keywords: *LC/MS/MS, Wine, Polyphenol, Tannin, Fraud*

B-28
DNA-BASED CALIBRANTS FOR QUANTIFICATION OF COFFEE ADULTERANTS

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Food authenticity is presently a subject of great concern for food consumers and authorities. In the last years, several methods based on polymerase chain reaction (PCR) have been proposed as useful tools for identification and authentication of food and feed, allergens and genetically modified organisms. Coffee is one of the most popular beverage across the world. As such, it has been a target of fraudulent admixtures with a diversity of cheaper agro industrial products as roasted barley, corn and rice. Nevertheless, the detection of such foods in roasted coffee may present difficulties due to the intensity of the thermal processing during coffee roasting. In order to ensure the purity of coffee, a highly sensitive and selective methodology for detection of adulterants was developed, based on DNA analysis. The first step in this work was to develop DNA calibrants for detection and quantification of barley, maize and rice in roasted and soluble coffee, since, there was no Certified Reference Material available. For this purpose, endogenous and adulterant calibrants were defined for the real-time PCR runs. In this way SYBR Green dye was performed using the designed primers COFFE1, BARLEY3, ZEIN2 and RICE1 with different concentration solutions of genomic DNA from each target to build calibration curves. PCR were carried out in decaplicate. The linear regression equations, correlation coefficients (R2), sensibility (LOD and LOQ) and reactions efficiency were determined. LOD for coffee, barley, corn and rice DNA standards were 0.7, 5.0, 0.1, and 0.9 pg, respectively, while LOQ were 2.4, 8.1, 0.3 and 2.6 pg, respectively. Efficiency levels for detection of each primer target set of coffee, barley, corn and rice were 88%, 95%, 104% and 107%. These results showed the production and evaluation of DNA-based calibrants for detection and quantification of major adulterants in roasted and soluble coffee.

Keywords: *Adulterants, real time PCR, roasted coffee*

B-29
LABEL-FREE DNA DETECTION AND QUANTIFICATION METHOD BASED ON FUNCTIONALIZED LPGS

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The rapid detection of base sequences of deoxyribonucleic acid – DNA is an incredibly active research field due to its numerous applications. Indeed this concept can be of use in disperse areas, e.g. food safety, food traceability, genetics, pathology, pharmacogenetics, criminology, civil defense, among others [1-3]. Nonetheless the present methods are quite limited, since they can only detect a given sequence, and it is necessary to mark it with a fluorophore. Moreover, these methods are limited by its detection ability, since they cannot accurately quantify the DNA necessary for hybridization to occur. The need for a fluorophore is also a disadvantage since the traditional fluorescence probes used are prone to photobleaching and chemical instability. Furthermore, the use of markers is quite expensive in terms of equipment and materials required [4]. The method here presented is a possible landmark in terms of sequence DNA quantification. It is based on the functionalization of an optical fiber long-period grating (LPG) with single strand-DNA (ss-DNA). Once the ss-DNA is immobilized in the LPG, the sensing system is put into contact with complementary, partial-complementary and non-complementary DNA in order to assure the occurrence of specific-hybridization. The results obtained for this sensor are quite interesting, since not only hybridization occurs with total complementary DNA, but it also allows the detection and quantification limits determination of the complementary-DNA required for hybridization to occur. Furthermore, both limits are quite low (Detection Limit: 62±2 nM and Quantification Limit: 209±7 nM). These low limits are followed by a very good sensitivity 77±2 nM. These results are quite important since they show that the amount necessary for hybridization to occur is quite small and well within the nan oscale range. Moreover it allows the implementation of more complete measurement systems, as it enables the detection of specific DNA strands which in some specific cases may be enough, and also quantifies with a high degree of certainty in the hundreds of nanomolar. This system is cheaper and simpler than the currently available methods for DNA detection and as far as the author's knowledge goes, these quantification and sensitivity results are the first to appear in this competitive area.

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Keywords: *DNA detection, optical fiber long-period grating (LPG), traceability*

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B-30
LPAS BASED DETECTABILITY OF METHANOL IN ALCOHOLIC BEVERAGES

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Methanol, also known as wood alcohol, is produced in very low concentration as natural by-product during the wine fermentation process, where the main alcoholic content is due to ethanol. Differently from ethanol, methanol has a strong specific toxicity for the human body (minimal lethal dose in adults around 1 mg/kg of body weight). The presence of methanol in beverages is not evident, because of its similarities to ethanol in both appearance and odor. The alcoholic composition is normally stated by chemical analysis. A fast detection of methanol may be based on high resolution infrared spectroscopy, which possess in principle the capability to record in detail the methanol spectral fingerprint. In this feasibility study, the Laser Photoacoustic Spectroscopy (LPAS) technique was applied to the analysis of alcoholic mixtures. Spectra of both alcohols were analyzed as pure substances as well as alcohol-water mixtures. Significant wavelength dependent differences were observed in term of relative absorption intensity, as expected. A high resolution spectral analysis was performed for mixtures of both alcohols in water as well, for different relative concentrations. The comparison among the recorded spectra was performed with the Principal Component Analysis (PCA) method. The experimental result is shown.

Keywords: *Methanol, vibrational spectroscopy, fraud, food safety*

Acknowledgement: *We acknowledge the financial support of Project SAL@CQO*

B-31
DISCRIMINATION OF VEGETABLE OILS USING NMR SPECTROSCOPY AND CHEMOMETRICS

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NMR spectroscopy is a very good alternative to botanical discrimination of vegetable oils using ^{13}C isotopic analysis and a possible alternative to conventional chromatographic methods for oil composition determination. In this study, over 50 samples, either pure or combined into binary blends of vegetable oils with different botanical and geographical origin have been analyzed by ^{1}H and ^{13}C NMR spectroscopy in order to discriminate between them, to determine their lipid composition and to detect the admixture ratio in the case of blends. The data were also modeled by chemometrics for accurate sample classification. The NMR data allowed a correct botanical origin identification of the vegetable oils, highlighting the discriminating chemical signals. Using this approach, blends of 1% v/v olive oil in sun-flower oil were detected. The results strongly support the capability of NMR and chemometrics to be used in quality assessment of vegetable oils in terms of their botanical origin.

Keywords: *Chemometrics, ^{13}C NMR, ^{1}H NMR, vegetable oils*

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B-32
FINGERPRINTING ANALYTICAL STRATEGY USED FOR AUTHENTIFICATION OF HONEY

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To find the most reliable methods for authentication of food or food ingredients has always been a challenging issue. Our research is of particular interest in the geographical discrimination of honey from different floral sources and different regions of Romania as support for quality assessment and classification activities. This study demonstrates both the benefits and the advantages of coupling different analytical techiques in order to ensure honey authenticity due to the fact that honey is a complex mixtures of molecules (toxins and peptides) and a challenging product to analyze. The control of quality and the assumption of the claimed botanical and geographical origin of honey, which is associated to the producing vegetation area, flowering period of the plants, are of prime importance for stakeholders from apiary industry in order to reinforce the consumer trust. Concerning the various possibilities of honey adulteration, peptide mass fingerprinting (PMF) and protein profiling methods have been used. This study reports also the use of isotope ratio mass spectrometry (IRMS) and site-specific natural isotopic fractionation measured by nuclear magnetic resonance (SNIF–NMR) for the assignment of origin and the proof of adulteration. The analyzed honey samples were from different geographical areas and different botanical origin. A fast, suitable and alternative technique to classify honey samples according to their geographical origin was developed based on PMF using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOFMS).

Keywords: *Authentication, fingerprinting, honey*

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B-33
STUDY PROTOCOL TO ASSESS THE WATER RETENTION IN POULTRY ORGANS AIMING ON THE CONTROL OF ECONOMIC FRAUDS

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The amount of retained water in meat products is a concern for consumers, not from a food safety perspective, but for economic implications. Apart from the natural water content, post-evisceration processes may increase the quantity of water, thus leading to an incorrect weigh of the actual product. The water-immersion chilling method, which is traditionally used by poultry processors, is essential to prevent potential food safety problems. In carcasses and parts, the Brazilian regulation controls the amount of water retained, by assessing the products in official laboratories. However, for organs, especially chicken heart, liver and gizzard, which are highly consumed in Brazil, such regulation is still being worked on. As requested by the inspection departments, a study protocol was prepared in order to assess the scenario of water retention in poultry organs. This project was designed in such a way that the most critical variables of the industrial process are assessed, with the objective of measuring the influence of each step in the final amount of absorbed water. In order to make it feasible, considering some shortcomings like the long distances between the origin of the sample and the laboratory, the size and workload of the plants and the variety of techniques used in the post-evisceration processes, the protocol was refined ending on a practical yet robust experiment, which shall give data in the quality and quantity expected.

Keywords: *Laboratory Network, poultry, fraud, water retention*

B-34
ASSESSING THE WATER RETENTION IN POULTRY AIMING ON THE CONTROL OF ECONOMIC FRAUDS

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In order to establish a regulatory limit for the amount of retained water in raw poultry products in Brazil, an integrated study was conducted by the Lanagros (National Agricultural Laboratories). In cooperation with the inspection services, a number of 652 samples were collected, performing 7760 analytical tests, from 6 states. A profile of the national production was then traced, considering the following variables: age of the birds, species, type of feed and weight. Also, the quantity of physiological water was distinguished from the absorbed water after chilling. Chicken breast, half breast, breast without skin, thigh, drumstick and thigh with drumstick were the products tested in the official laboratories, following the same method. The present work details that experiment, showing an effective example of how a laboratory network supports the inspection services in their actions, not only with analytical reports, but also performing studies and giving t echnical basis for new legislation.

Keywords: *Poultry, water retention, fraud, laboratory network*

B-35
VALIDATION OF PCA-ASSISTED ANALYTICAL METHODS FOR FOOD AUTHENTICITY CONTROL

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Principal components analysis (PCA), one of the most frequently applied statistical techniques to reduce dimensionality, has become increasingly important in recent years in food authenticity control. Meanwhile there are plenty of publications in which spectroscopic, chromatographic or mass spectrometric data are submitted to PCA and the resulting two- or three-dimensional plot is used for visual differentiation between authentic and non-authentic samples. For practical purposes in routine analysis, two things are necessary. First, a statistically defined criterion by which one can decide whether a sample is to be classified as authentic or as non-authentic. Second, the lowest degree of adulteration that may, with reasonable certainty, be expected to lead to detection of non-authenticity. An approach to the determination of these method characteristics from empirical data under assumption of multivariate normality is described and derived in detail in [1]. This contribution illustrates, by means of an example, how an analytical method in combination with PCA data processing can be employed in the routine practice of food authenticity control and how such a test method can be validated.

[1] P. Steliopoulos (2013) J. Verbr. Lebensm. 8: 71–77.

Keywords: *Principal components analysis, food authenticity*

B-36
ANALYSIS OF WOOD RELATED CHEMICAL MARKERS IN AGED WINE DISTILLATES BY CAPILLARY ZONE ELECTROPHORESIS

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Ageing is one of the most important factors determining quality of wine distillates and divins. The relevance of the research concerning distillates quality caused by the fact that they are the raw material for the production of divin, representing aged brandy, produced in Moldova in conformity with the classic technology of cognac production. Identification of distillates is complicated by complex chemical composition, resulted from the interaction between the components of the wine distillate and oak barrel. Composition of the distillates influencing their quality characteristics, especially taste and aroma, are caused by decomposition of wood macromolecules and subsequent extraction, reactions between wood components and constituents of the raw distillate, reactions involving wood extractables, reactions involving distillate components, evaporation of volatile compounds. In the present work, it has been studied the relationship between the age of wine distillates as the main indicator of their quality and accumulation of lignin degradation products, such as aromatic aldehydes and acids. The wine distillates with ageing times from 5 to 22 years from different producers were investigated by capillary zone electrophoresis under the following conditions: silica capillary with an effective length of 60 cm and an internal diameter of 75 µm, voltage of 25 kV, detection at 373 nm. 0.02 M sodium tetraborate with a pH of 9.2 was used as buffer solution. The total duration of the analysis was less than 6 minutes. Despite the differences in absolute values in the content of aromatic aldehydes and vanillic acid between the samples from different manufacturers, some correlations were detected. The total amount of the oxidation products of sinapil alcohol (sum of sinapaldehyde and syringaldehyde), and coniferyl alcohol (sum of coniferaldehyde, vanillin and vanillic acid) increased with increasing ageing time. The presented analysis method involves a minimal time, costs and reagent consumption, providing a t the same time good separation efficiency, and it is ideally suited for rapid analysis of aged wine distillates to determine their overall quality, approximate ageing time and signs of counterfeiting.

Keywords: *Distillate, electrophoresis, aromatic aldehydes*

B-37
A MULTIRESIDUE METHOD FOR THE SIMULTANEOUS DETERMINATION OF ILLEGAL DYES IN EGGS BY LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

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It is an old practice that producers enhance their products' colour to make it more attractive for consumers. Also the eggs' nutrition values and freshness in many societies is highly linked with the egg yolk colour. Those with yellow-orange colour are the most wanted among the consumers. Due to that, the egg producers' colour of their products by feeding the laying hens with feed containing the dyes (natural or synthetic). In commercial food production there are numerous of dyes approved for use, registered as feed additives. But there are also reports of food adulteration by using unauthorised dyes, which are often very hazardous. These substances include among others Sudan dyes, which were detected in 2006 in eggs. This work presents a method for the simultaneous determination of dyes unauthorised for use in laying hens (Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Red G, Sudan Orange G). Some of them are banned as food additives but very often detected in such products as: curcuma, red pepper or curry and others are allowed as food additives but forbidden for use to enhance the egg yolk colour. Because the dyes are soluble in fats and most of the fat content in eggs is located in the egg yolk – this compartment was used to develop the sample pre-treatment step. Dyes were extracted using 1% formic acid in acetonitrile and cleaned using zirconium coated silica columns. After dilution with 0.1% formic acid samples were analysed using LC–MS/MS system with acetonitrile (A) and 0.1% formic acid (B) as a mobile phase in a gradient mode and core-shell analytical column. Method was validated according to requirements described in the Commission Decision 2002/657/EC: linearity, precision (repeatability and within-laboratory reproducibility), recovery, decision limit CCα and detection capability CCβ was calculated. Within-laboratory reproducibility was in the range of 10–25%, with recovery above 65%. Developed method fulfilled all performance criteria an can be used in the official survey of dyes residues in food of animal origin.

Keywords: *Sudan dyes, LC-MS/MS, eggs*

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B-38
NOVEL REAL-TIME DATA MINING FOR GC–TOFMS: THE FIGHT AGAINST FOOD ADULTERATION

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Food aroma profiles typically contain a complex mixture of components with wide-ranging concentrations and odour threshold values. A detector with high sensitivity and fast spectral acquisition is therefore essential to maximise analyte detection and identification. Gas chromatography coupled with time-of-flight mass spectrometry (GC–TOFMS) provides the ideal solution for the analysis of such samples. However, the high throughput analysis afforded by TOFMS also requires comprehensive data analysis to make full use of the acquired data. The ability to provide real-time dynamic baseline compensation to remove background ions (e.g. from column bleed or solvent tails) and real-time spectral deconvolution allows immediate perception and qualification of target compounds. Moreover, the deconvolution yields lists of reliable orthogonal ions that can quantify targets – dynamically and without skew - in situations of extreme co-elution or where the analytes are heavily masked by matrix. Harmonising organoleptic assessment of product aromas with chemical analysis is often a great challenge in production line quality assurance. This demands the rapid identification of adulterants or odour-taints which, while easily picked out by a human sensory panel, may contain compounds with low odour thresholds, existing at trace concentrations that cannot be chromatographically resolved from the matrix. Novel software tools, described in this work, simplify the comparison of complex chromatograms, allowing minor differences to be readily and automatically distinguished. MS chromatographic data, following their deconvolution and normalisation, are converted into histogrammed transforms, where target components are represented as precise centroids of abundance in a time-independent domain. A closest-fit can be generated between reference and sample chromatograms, or between two classes, or for matching an unknown sample against a database of reference mixtures, removi ng any subjectivity associated with comparative analysis. Specifically for aroma profiling, where the sensory differences may lie in the minutiae of analysis, a non-linear compression function in the comparison algorithm enables discrimination to be maximised without loss of data. This poster will describe the combined use of such novel data processing features for the robust characterisation of aroma profiles.

Keywords: *Quality control, adulterants, odour taints, GC–TOFMS, comparative analysis*

B-39
COMPARISON AND DISCRIMINATION OF BLUE, WHITE AND OCHER POPPY SEEDS ACCORDING TO ELEMENTAL AND AMINO ACIDS PROFILES

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In the European region, poppy seeds are mostly used in confectionery and in baking to filling of products, and possibly to sprinkling on rolls and bread. In the past, the blue poppy seed varieties were the most used in food industry, but currently new varieties of ocher and white poppy seeds were bred, being further cultivated. These varieties would be applicable in producing confectionery due to their nutty flavor. Therefore, our work is focused on the detection, comparison and discrimination of toxic, hazardous, nutritional elements, and amino acids contents in respective poppy seeds varieties. The present study was performed with the aim to compare elemental and free amino acids compositions in blue poppy seeds of Bergam, Gerlach, Major, Malsar, Maraton. Opal, Orfeus, Aristo, Buddha, MSZB- 3, MS – 387 and MS – 423 varieties, as well as white poppy seeds of Albin and Racek varieties, and ocher poppy seed of Redy variety, all growing in Slovakia in the region Malý Šariš. All the samples were analyzed for determination of toxic elements Cd, Hg, Pb, the risk element Mo and nutritional elements Ca, Cu, Fe, Mg, K, Na, Zn by means of AA spectrometry method using the Perkin Elmer 4100 equipment with HGA 700. Twenty types of amino acids also were investigated by LC/ESI–MS–MS chromatographic method using the Agilent 1200 equipment with Agilent 6410 Triple Quad detector and ESI interface. All varieties of poppy seeds were statistically compared to the content of selected elements using ANOVA statistics (Multiple Comparisons). The contents of Hg, Cd, Cu, Zn and Ca were significantly different in all of examined colour poppy seeds ($P < 0.05$); the contents of Fe and K were significantly different only in white and blue poppy seeds. Regarding Mg, its content was significantly different only in blue and ocher poppy seeds. Molybdenum content did not show any distinction within coloured poppy seeds varieties. Concerning the free amino acids contents: there is no significant difference among selected varieties. Differentiation of particular poppy seed varieties with respect to the colour of seeds was effectively accomplished by use of multivariate statistical discriminate analysis. Canonical discriminant analysis was selected, in which Zn, Fe, Ca, Hg, and amino acids aspartic acid and glycine were determined as the most effective discriminators. As a result, 100% classification of tested samples was achieved according to elements and amino acids contents.

Keywords: *Poppy seeds, elements, AAS, amino acids, LC/ESI–MS–MS*

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B-40
CAN LCMSMS BE USED IN HORSE MEAT DETECTION?

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Following the UK Food Standards Agency (FSA)'s announcement in January that horse and pig DNA had been identified in beef products sold by several supermarket chains, further testing across Europe and beyond has revealed widespread incidences of such food contamination. However, most testing methods are based on detection of species-specific DNA in meat, using the polymerase chain reaction (PCR) – which does not detect or identify proteins. This is a concern because DNA can be easily disrupted or removed during standard meat processing and food manufacturing. As a result, horse tissue or other contaminants remain undetected in food samples, despite strong presence of the contaminating proteins. An alternative protein-based method, ELISA (enzyme-linked immunosorbent assay), can be used to complement DNA testing, but this method has limitations, including that it detects only one part of the protein and not multiple protein markers. The LC–MS/MS-based met hod presented offers a more accurate and reliable approach to meat speciation than PCR or ELISA-based techniques or other indirect methods, and also allows for the detection of veterinary drug residues in the same analysis, which is not possible by ELISA or PCR. The method is developed using a micro LC system coupled with to LC–MS/MS system and uses multiple reaction monitoring (MRM) to detect peptide markers for horse and is capable of providing sequence information by acquiring a product ion scan for each triggered MRM which can be used to further confirm the peptide's / proteins and therefore the species identity. Using the same extraction and LCMSMS method it is also capable of simultaneously of detecting veterinary drug residues by adding additional MRM experiments. The method has been shown to be capable of simultaneously detected phenylbutazone below 10 µg/kg as well as a 1% contamination of horse meat in beef. This approach offers food analysts the ability to detect multiple species and veterinary drug residues in a single approach which is not possible by any other technique to date.

Keywords: *Meat, Speciation, LC–MSMS, Veterinary, Residues*

B-41
METABOLIC PROFILING IN ASPERGILLUS FLAVUS TO DETERMINE GENE FUNCTION

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The Aspergillus flavus metabolome is of considerable interest because A. flavus produces the most toxic and carcinogenic compounds known from fungi and is a source of world-wide episodes of contamination of foods and animal feed with aflatoxins. Examination of the genes predicted to be involved in secondary metabolite biosynthesis suggests that A. flavus is capable of producing many more metabolites than have so far been discovered. We have begun a collaborative project to examine some of these gene clusters to determine both their capability of producing known as well as unknown metabolites. To date, we have closely examined metabolite production by wild type A. flavus and mutants in biosynthetic genes in Clusters 11, 23, 27 and 41. The backbone genes in clusters 27 and 41 are predicted to encode polyketide synthases (PKS). Cluster 11 contains a non-ribosomal peptide synthase (NRPS), while the backbone gene of cluster 23 encodes a hybrid PKS/NRPS. Using a comparative metabolomics approach based on UHPLC/Orbitrap MS, we have been able to detect metabolites that are unique to each of the clusters under investigation. Our results show that Clusters 11 and 27 produce the known A. flavus metabolites ditryptophenaline and asparasone, respectively. The PKS/NRPS of Cluster 23 produces a tennellin-like metabolite, while the PKS of cluster 41 is predicted to be a MSAS synthase that is most likely after conversion to a meroterpenoid is necessary for developmental signaling. Such metabolites have been characterized in A. nidulans and other species. The LC/MS and MSn data are consistent not only with the products expected from these cluster backbone genes but also with the modifiying enzymes encoded by decorating genes in each cluster.

Keywords: *Aspergillus flavus, Aflatoxin, fungal secondary metabolites, comparative metabolomics, high resolution mass spectrometry*

B-42
PROFILING OF WHISKIES FROM DIFFERENT ORIGINS WITH ACCURATE MASS LC/MS

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Single malt whisky samples from different geographical origins were analyzed by electrospray LC/MS, using an accurate mass TOF instrument (PerkinElmer AxION 2 TOF), using a UHPLC reversed-phase separation with negative mode detection. This method detects a large number of low volatility phenolic compounds, which originate from malted barley, and are adsorbed from charred oak wood during cask maturation. Samples were analyzed in random order, to reduce the contribution to the statistical analysis of any systematic drifts over the time course of the study, and a number of replicate analyses were acquired for each sample. Datasets were processed to reduce the complexity of the full spectral and time information and yield significant (mass, time, intensity) co-ordinates. These details were correlated for all the datasets, allowing for minor changes to both masses and retention time between analyses, to product a table of all the (mass, time) details detected in each of the datasets, with their respective intensities. The complete table was imported into TibCo SpotFire (PerkinElmer) to develop methods to differentiate the samples. Various chemometric models were investigated, such as hierarchical clustering and principal component analysis. Clearly differentiated sample datasets revealed (mass, time) markers which were significantly different in intensity. The accurate masses and isotopic patterns of these marker ions were used to obtain candidate elemental formulae, which were correlated to chemical compounds in the whisky. The marker compounds relate to the geographical origin and maturation processes for different whiskies. These statistical models will allow additional samples to be added to the sample cohorts over time, to further refine the models and build up more complete information of the typical components of different whiskies. Such models may be extended to allow detection of adulterated whiskies which do not fit to the component patterns of known single malt and blended whiskies.

Keywords: *Chemometrics, whisky, LC/MS*

B-43
CHARACTERIZATION OF VOLATILE FINGERPRINTING AND QUALITY OF MUSCAT- AND NEBBIOLO-BASED WINES USING HEADSPACE SOLID-PHASE MICROEXTRACTION COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY (HS–SPME/GC×GC/TOF–MS)

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The evaluation of wine volatile flavor is a key tool for an "objective" chemical description of the aroma. Quality of wines is correlated to the volatile compounds of the aroma. Despite the complexity, volatile aroma can lead to more information about natural compounds in wine (derived from grape, from microbial fermentation or from biochemical changing during ageing as well as more information on environmental/biological volatiles or semi-volatiles "in trace" contaminants (e.g. alo-anisoles). GC×GC technique is today a consolidated performing analytical strategy allowing the separation of complex mixtures of volatiles organic compounds. GC×GC approach, largely applied to some complex matrices like roasted foods (e.g. coffee, cocoa, nuts) is currently under-exploited in the field of the "wine aroma fingerprinting". Selectivity of orthogonal 2D separation, when coupled with TOF–MS detector, allows obtaining a powerful tool useful to describe the u ntargeted fingerprint of wines. Aim of this communication is to show some results recently obtained on Muscat- and Nebbiolo-based wines from Piedmont (Italy). A headspace solid-phase microextraction (HS–SPME) method, coupling comprehensive two-dimensional gas chromatography (GC×GC) time-of-flight mass spectrometry (TOF–MS), was optimized in order to study i) the evolution of the aroma in Muscat wine stored in different conditions and ii) the aromatic profile of Nebbiolo-based wine, also concerning the identification of in trace off flavors (alo-anisoles). Moreover, iii) an original new post-analytic approach to 2D contour plots comparison has been developed in order to automatically evaluate – after normalization - the identification of any differences among key volatile compounds, calculated from pixel-to-pixel re-designed 2D chromatograms. This study demonstrates an important advancement in wine volatile analysis, as the method allows for the simultaneous analysis of a significantly larger number of compounds found in the wine headspace, when compared to other current single dimensional GC–MS methodologies. This approach allowed for the simultaneous analysis of over then 120 tentatively identified volatile/semi-volatile compounds found in the Nebbiolo wine headspace. The aroma patterns of Asti Spumante and Moscato d'Asti wines, stored in bottles for 6 months at two temperatures, were assessed. Wines stored at 5°C did not show significant changes in flavor; otherwise, the samples stored at 15 and 25°C, showed a significant decrease in linalool, b-damascenone, ethyl hex- anoate, and ethyl octanoate levels. Alpha-terpineol, hotrienol, nerol oxide, furanic linalool oxides A/B and rose oxide concentrations significantly increased in samples stored at 25°C. Concluding, this approach should be considered an interesting advanced method to fingerprint aroma, in order to comprehensively characterize wines, under the metabolomic profile.

Keywords: *GC×GC–TOF, flavour, wine aroma, Muscat, Nebbiolo*

B-44
DETECTION OF COW MILK ADDED TO MORE EXPENSIVE MILKS FOR ECONOMICALLY MOTIVATED FRAUD BY LC–MS

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Milk adulteration by small molecules for economically motivated fraud is well known, and methods for detection have been examined extensively using, amongst other tools, LC/MS techniques. Current techniques tend to focus on small molecule adulteration; however, large molecule adulteration is becoming increasingly common and is not yet regulated within the dairy industry. One of the simplest forms of milk adulteration is to dilute down more expensive milks from species such as sheep and buffalo with less expensive cow milk. We have developed a method for measuring the addition of bovine milk to more expensive milks (goat, sheep, buffalo and camel) by using liquid chromatography coupled to electrospray time-of-flight mass spectrometry (LC–TOF–MS) to detect species-specific marker proteins in the milk. Milk contains lactoglobulin, lactoferrin and casein proteins at high concentrations; the water soluble proteins left in the whey fraction after a liquid/liquid extraction [1] contain a high abundance of beta –lactoglobulin B (18 kDa), with some species also having beta –lactoglobulin A. The amino acid sequence and mass of beta-lactoglobulin B is species specific. The high resolution, high mass accuracy of TOF–MS allows for detection of a protein mass within 1Da of the calculated mass. Measurement of the whey proteins allows for unambiguous confirmation of low levels of bovine lactoglobulin B (18,277 Da), even in the presence of other lactoglobulins, such as goat lactoglobulin B (18,187 Da) Milk samples from different animals were spiked with varying levels of bovine milk and homogenized. Following liquid/liquid extraction, the proteins in the whey fraction were then separated using a fast gradient HPLC method, followed by detection with an AxION 2 TOF mass spectrometer (PerkinElmer). Adulteration of less than 5% by volume with cow milk was visible from the lactoglobulin protein levels.

[1] Chen et. al., Rapid Commun. Mass Spectrom. 2004; 18: 1167–1171

Keywords: *Milk, proteins, LC/MS*

B-45
RAPID MEASUREMENT OF POMEGRANATE JUICE ADULTERATION WITH MINIMAL SAMPLE PREPARATION USING DSA/TOF

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Rapid measurement of pomegranate juice adulteration with minimal sample preparation using DSA/TOF Food adulteration or food fraud occurs when an ingredient is replaced partially or fully with something different – without the knowledge of consumer. Most of time, food adulteration occurs to improve profits but in some cases, there can be serious health consequences. Fruit juices are among the top 10 adulterated foods. In the past, methods employing gas chromatography- mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) hyphenated to MS (HPLC–MS) have been implemented for measuring food adulteration. These methods are time consuming, expensive and require extensive sample preparation. Recently, we have developed DSA (Direct Sampling Analysis) ionization source which utilizes field free APCI source. The advantages of this method, compared to conventional techniques, are direct sampling with minimal or no sample preparation with high throughput. In this work, DSA coupled with high resolution and accurate mass capabilities of TOF–MS were used for me asurement of adulteration of pomegranate juice with grape juice and apple juice. In literature, it has been reported that tartaric acid is present in grape juice but absent in pomegranate juice and malic acid levels are about 10 times higher in apple juice in comparison to pomegranate juice. Therefore, using DSA–TOF, we demonstrated that the presence of tartaric acid and elevated levels of malic acid in pomegranate juice can be used for measuring its adulteration with grape juice and apple juice with minimal sample preparation and measurement time less than 30 s.

Keywords: *Rapid food analysis direct sample*

B-46
POTENTIALS AND CAVEATS WITH OXYGEN AND SULFUR STABLE ISOTOPE ANALYSES IN AUTHENTICITY AND ORIGIN CHECKS OF FOOD AND FOOD COMMODITIES

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The analysis of stable isotope ratios is an officially accepted method in food authenticity and origin determination. It is routinely performed by empirical comparison of the unknown samples' δ values with those of authentic material. However, as the isotope characteristics of food are influenced by many parameters, like primary material, thermodynamic and kinetic isotope effects, geographical origin and composition, it is desirable to also use causal correlations of isotope fractionations for the interpretation of experimental data. This is demonstrated for the stable isotopes of oxygen and sulfur.
In the natural water cycle, plant leaf and animal cell water are the most important sources for food integrated water and organically bound oxygen. Equilibrium isotope effects on phase transitions and oxygen exchange reactions of water determine the oxygen isotope characteristics of bulk foodstuff and ingredients, permitting their assignment to geographical origin, history and authenticity. This is demonstrated for juices, wine, meat, and eggs. However, it has to be taken into account that the isotopic equilibration between water and organic functionalities is a slow reaction, and that animal cell water is a product from many precursors. A second source of organically bound oxygen is atmospheric O_2. A kinetic isotope effect on mono-oxygenase reactions leads to $\delta^{18}O$-values of +7 ±1‰ e.g. of phenolic OH-groups. This permits the assignment of L-tyrosine to plant and animal origin, basis for the identification of trophic levels and the proof of illegal meat and bone meal feeding to cattle, and it explains the ^{18}O-enrichment of collagen relative to other proteins from the same source.
Sulfur occurs in Nature in different forms, oxidation states and $\delta^{34}S$-values. The most important primary S-sources for plant and animal organic matter are soil/water sulfate and atmospheric SO_2 with characteristic $\delta^{34}S$- and $\delta^{18}O$-values. Isotope ratio determinations for origin assignment, trophic level and animal migrations studies always use the *bulk* $\delta^{34}S$-value. Examples are the origin identification of asparagus and of lamb meat and the assignment of fish to sea and fresh water breeding, respectively. However, as the assimilatory sulfate reduction is accompanied by isotope fractionation, in plants and plant organic compounds (e.g. glucosinolates), sulfur in the oxidized form is generally relatively enriched, in the reduced ("organic") form depleted in ^{34}S. Even between defined organic compounds from the same origin, $\delta^{34}S$-value shifts can occur, and differences of their concentrations in defined tissues can simulate isotope fractionations. This is demonstrated for connective tissue (cartilage) with relatively ^{34}S-depleted proteoglykanes and -enriched collagen.

Keywords: *Oxygen, Sulfur, Stable Isotopes, Authenticity, Origen Check*

B-47
DISCRIMINATION OF MINOR COLD PRESSED OILS BY FLAVOR ANALYSIS

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Flavor analysis is used to get the information about complex food matrices in the field of food quality and safety, its authenticity, freshness or flavor values accepted and/or looked for by consumers. The aim of the study was to evaluate the flavor of minor vegetable cold pressed oils using sensory analysis and instrumental measurements by means of Solid Phase Micro Extraction (SPME) technique coupled with GC-MS and then to discriminate them by chemometric analysis. Sensory analysis was performed by expert panel using Quantitative Descriptive Analysis (QDA) method in 2 sessions. Volatile compounds were determined by GCMS-QP 2010 Shimadzu using capillary column coated with polar phase ZB-WAX Plus. Chemometric analysis was performed with Principal Component Analysis (PCA) and Cluster Analysis (CA) using STATISTICA 10.0 PL. Cold pressed oils obtained from linseeds, camelina seeds, pumpkin seeds, borage seeds and evening primrose seeds were sampled. The relatio nships between volatile compounds and sensory attributes were discussed. Pumpkin seed oil was characterized by the highest values of positive sensory attributes (pumpkin, sweet, olive, nutty). In the head-space of linseed oil 38 volatile compounds were characterized, in camelina oil – 41, in pumpkin seed oil – 38, in borage oil – 42. Evening primrose oil had the lowest number of identified volatile compounds – 26. The main volatile compounds were aldehydes (pentanal, hexanal, nonanal), ketones (3-octanone, 2-heptanone, 2-octanone), alcohols (1-hexanol, 1-pentanol, heptanol) and organic acids (acetic acid, propionic acid, hexanoic acid). Aroma profiles of borage and pumpkin seed oils were characterized by presence of terpenes. Study showed that it is possible to discriminate oils by their aroma profile and also chemometric analysis showed means of classification of oils samples according to sensory attributes and volatile compounds. There is a possibility of employm ent of instrumental and chemometric analysis for authenticity and traceability tests of vegetable oils.

Keywords: *Cold pressed oils, volatile compounds, flavor analysis, chemometric analysis, GC–MS*

B-48
NEAR-INFRARED REFLECTANCE FOR THE RAPID PREDICTION OF QUALITY PARAMETERS OF QUINOA (*CHENOPODIUM QUINOA*)

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Quinoa (*Chenopodium quinoa Willd*.), a native Andean crop, was an important staple in the Incan civilization, who replaced the animal protein in their diet with quinoa. This pseudocereal is still widely cultivated in South America, including Bolivia, the largest producer with 20,000 ha per year, followed by Peru, Colombia, Ecuador, Chile and Argentina. It is a valuable source of quality protein, carbohydrates, essential fatty acids, micronutrients and also gluten-free [1]. The official methods to measure the quality parameters are time-consuming, destructive and generate chemical residues. By the other hand, near-infrared reflectance spectroscopy (NIRS) is a rapid non-destructive technique that is able to measure chemical properties [2]. However, the use of NIRS must be associated with chemometrics tools to predict parameters as moisture, ash, lipids, proteins and carbohydrates. The partial least squares (PLS) algorithm is used to build the calibration model by regressing the spectra against the reference value obtained in laboratory. Considering the advantages of NIRS technique, the objective of this research was to develop NIRS calibration model suitable for the routine determination of dietary constituents in quinoa varieties using PLS, moreover the application of whole grain and quinoa flour to construct the calibration models was compared. The total moisture, ash, lipid, protein and carbohydrate contents were determined using reference methods described by the AOAC (2011). The data were obtained using a PerkinElmer 400N FT-NIR spectrometer and chemometric analysis was performed in MATLAB 7.8 with PLS-toolbox 5.8. The samples were separated in two groups: calibration (68) and validation (30) sets. Validation was carried out both by means of cross-validation and test set validation. Various spectral treatments were employed to correct baseline shifts arising from scattering: constant offset elimination, first and second deriv ate, standard normal variate (SNV) and straight line subtraction. For both samples group, flour and whole grain, the PLS models developed for quantification of all quality parameters showed that the proposed methodology produced suitable results with the graph of the real and predicted concentrations with coefficient of determination > 0.68 and RMSEP < 4.56 %. Therefore, the viability of the NIRS technique with no waste generation, low cost, reduced time and no sample preparation for replacing laborious methods of analysis was demonstrated because the results for grain were satisfactory.

Keywords: *Near-infrared reflectance spectroscopy, Chenopodium quinoa, multivariate analysi*

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B-49
IDENTIFICATION AND QUANTIFICATION OF ROE DEER (*CAPREOLUS CAPREOLUS*) IN FOOD FOR DETECTION OF FOOD ADULTERATION

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According to legal regulations, food manufacturers have to ensure not only the safety but also the authenticity of food products. However, to increase their profit, manufacturers may be tempted to incorrectly label their products and to use lower priced ingredients of inferior quality instead of more expensive ones. In the meat industry, game meat is particularly susceptible for fraudulent labelling since game meat has always been especially appreciated due to its distinctive flavour and its low fat and cholesterol content. According to the Codex Alimentarius Austriacus, in sausages declared as “game sausages” at least 38% of the total meat content has to be game meat. Analytical methods have to be specific and sensitive in order to be applicable for the detection of food adulteration. The real-time polymerase chain reaction (PCR) is a DNA based method that does not only allow the identification but also the quantification of species, e.g. meat species in foods. Quantification of meat species is, however, known to be a difficult task. The quantification strategy is usually based on a reference gene that is found in all animal species. The aim was the development of a real-time PCR method targeting genomic DNA sequences to identify roe deer (Capreolus capreolus) and to quantify its meat content in commercial food products. The challenge for the development of the method was the design of specific primers and probes. No cross-reactivities with other animal species, especially closely related game species, and spices that are often found in processed foods, should be achieved. In the present study, a TaqMan real-time PCR method for the identification and quantification of roe deer in game meat products was developed and validated. The PCR method was found to be specific for roe deer and does not show any cross-reactivities with other game species (e.g.: red deer, sika deer, fallow deer, wild boar and reindeer), other animal species (e.g.: pork, cattle, chicken and horse) and spices. The analysis of meat mixtures from roe deer and pork showed that the real-time PCR method is applicable to quantify the game mea t content in foodstuffs.

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Keywords: *food authentication, game meat, capreolus capreolus, real-time PCR, quantification*

B-50
EXPLOITING DNA MARKERS FOR THE BOTANICAL ORIGIN IDENTIFICATION OF HONEY

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Honey is a natural product highly consumed due its known association with health benefits. Monofloral and Protected Designation of Origin (PDO) honeys have generally higher economic value since they are considered to have higher quality due to its specific and well-defined flavor and aroma. Thus, these products are susceptible to misleading labeling and fraudulent practices, making their assessment of botanical origin and authentication a task of utmost importance. For this purpose, traditional methods based on pollen identification by microscopic analysis are presently used. However, this is time consuming and greatly depends on the experience and skill of trained analysts [1]. Recently, due to its high specificity and sensitivity, DNA-based methods are emerging as alternative tools for food authentication since they allow the unequivocal species identification. In this sense, the aim of this work is to extract amplifiable pollen DNA from honey for further exploiting molecular markers for botanical authentication. Considering the complexity of honey matrix, four extraction methods were tested and optimized: the commercial kits NucleoSpin® Plant II and DNeasy® Plant Mini Kit; and the in-house CTAB-based and Wizard methods as described by Mafra et al. [2] with modifications. Prior to DNA extraction, three different pretreatments were tested, accounting for a total of twelve protocols, which were applied to four different honey samples (Ericaceae, Rosmarinus officinalis and Eucalyptus spp. and multifloral). DNA extracts were evaluated by UV spectrophotometry to determine yield and purity. The amplifiability was tested by polymerase chain reaction (PCR) targeting rbcL gene, as a candidate locus for DNA barcoding. The obtained honey extracts revealed low DNA yields for all the extraction protocols, but adequate purity for PCR was achieved using the Wizard method. The amplifications were successfully attained with the Wizard metho d with one of the pretreatments when applied to all honey samples, while the other three methods exhibited lower reproducibility of results and low DNA yields regardless the applied pretreatment. The obtained results suggest the high potentiality of DNA-based methods to assess honey authenticity and the possibility to propose more robust, reliable, simple and sensitive assays alternative to the classical method for analyzing the botanical origin of honey.

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Keywords: *Honey, authenticity, DNA markers, PCR*

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B-51
RAPID GC–MS DETERMINATION OF ETHANOL IN SPIRITS

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The Commission regulation (EC) No 2870/2000 laying down the Community reference methods for the analysis of spirits (e.g. pycnometry, gas chromatography coupled to flame ionization detector (GC–FID)). Although the theory of these methods are very simple, the technique is time consuming and requires an experienced technicians, making it expensive and susceptible to error and there is a risk of results overestimation due to presence of the other volatiles. The aim of this study was to develop a fast, simple and effective method for the determination of ethanol using gas chromatography coupled to mass spectrometry (GC–MS) and isotopic dilution (ethanol-2,2,2-d_3) because this approach is more selective than classical routine methods. Using this novel approach, five samples may be prepared and analysed in approx. one hour, thus significant time saving were achieved compared to routinely methods. In addition, the method employs the significant reduce sample volume (50 µl) in comparison with pycnometry (100 ml of sample is needed at least) and the temperature control is not so critical. The validation of the new method was carried out on five different samples differing in ethanol content and other volatiles (pear brandy, grappa, havana, whiskey and vodka). Under the optimised conditions, differences between results obtained by GC–MS and pycnomtery were within 0.1–0.6 of ethanol content (%, v/v) and repeatabilities of the analytical procedure (expressed as relative standard deviation) were in range from 0.19% to 0.67% at six replicates. This newly developed rapid method is suitable for forensic analysis, particularly during scandals with adulteration of spirits (e.g. methanol scandal in Czech Republic in 2012–2013) due to control the maximum levels of volatiles expressed in grams per hectoliter of 100% vol. alcohol according regulation (EC) No 110/2008.

Keywords: *Ethanol; spirits; isotopic dilution; volatile compounds; GC–MS*

Acknowledgement: *Finacial support from specific university research (MSMT No 20/2013).*

B-52
HS–SPME–GC–TOF MS METHOD FOR TESTING OF ORANGE JUICE AUTHENTICITY

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Bottled orange juice, one of the most consumed non-alcoholic beverages, can be made of fresh oranges or reconstituted from orange juice concentrates. The difference in price between fresh and reconstituted juices can lead to intentional mislabeling, when reconstituted juice is being sold as fresh. During the concentrate production via the distillation of fresh juice, the volatile aroma compounds, which evaporate together with water, are trapped and kept separately from the concentrate. Before bottling, the juice is reconstituted using local water and flavoring agent. The concentrate and flavoring agent usually come from different oranges. The aim of this study was to prove the suitability of head-space solid-phase microextraction coupled to gas chromatography - mass spectrometry for fresh orange juice authenticity testing. The optimized method enabling the analysis of volatile aroma compounds was tested on various samples *(i)* fresh juice prepared from oranges in laboratory, *(ii)* fresh frozen juices delivered by our commercial partners *(iii)* pasteurized fresh and *(iv)* pasteurized reconstituted juices from the retail market. Using the Statistical Compare feature of the ChromaTof software by LECO, we were able to align the analytes of all measured samples and after the normalization of their areas, the statistical analysis was performed with promising results. The fresh bottled juices separated clearly from the group of reconstituted and from those delivered by our partners and our lab-made juices.

Keywords: *HS–SPME–GC–TOF–MS, volatiles, orange juice, authenticity*

Acknowledgement: *Financial Support from Specific University Research (MSMT No. 20/2013).*

B-53
CRITICAL ASSESSMENT OF METABOLOMIC PROFILING OF WINE VOLATILES EMPLOYING MASS SPECTROMETRY

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Wine, being a popular and, at the same time, expensive beverage is often exposed to fraud – the mislabeling of the origin or the variety of grapes is common. Effective tools for the testing of wine authenticity are necessary to disclose such practice and protect consumers. The approach we tested was based on measuring wine volatiles using head-space solid-phase microextraction coupled to gas chromatography – mass spectrometry. Both, the unit and high resolution mass spectrometers were used (TruTOF + Pegasus HRT, LECO, USA). Although the deconvolution & peak find algorithms were already successfully used and presented for the unit resolution MSDs, the application employing the ultra-high resolution is still unique. Compared to the unit resolution MSDs, the number of mass traces (i.e. single masses) is enormously higher when using high resolution MSDs. The ChromaTOF HRT software (LECO, USA) can, however, easily handle this data, as was demonstrate in our study. Using the optimized method, we analysed a set of 20 wine samples bought in specialized wine shops in the Czech Republic. The set of samples contained altogether 4 varieties. Our aim was to differentiate between the varieties based on the evaluation of measured volatile profiles. Statistical analysis (PCA, PLS-DA) was performed on the nominal mass resolution data in SIMCA 13.0 software. Analytes were aligned using the Statistical Compare feature of the ChromaTof software by LECO and their areas were then normalized. The volatile compounds evaluated as important markers were afterwards identified using the high resolution TOF instrument. Based on volatile profiles, samples tend to form groups, according to the variety of wine.

Keywords: *SPME GC/HRTMS, wine, volatiles*

Acknowledgement: *Financial Support from Specific University Research (MSMT No. 20/2013).*

BIOTECHNOLOGY BASED METHODS

(C-1 – C-4)

C-1
DEVELOPMENT AND OPTIMIZATION OF LIQUID CHROMATOGRAPHY – HIGH RESOLUTION MASS SPECTROMETRIC (U–HPLC–HRMS) METHOD FOR ASSESSMENT OF POLYHYDROXYALCANOATES (PHA) IN PSEUDOMONAS BACTERIAL BIOMASS

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Nowadays, using of bioplastics considered as an alternative to utilization of conventional plastic materials continues to increase. This is mainly because of their natural origin and biodegradability, which could perform a new approach to disposal of waste. In this terms of view, polyhydroxyalcanoates (PHA) originating as storage inclusions of several types of bacteria, seem to be very promising. Innovations in the area of biotechnology and gene engineering bring new challenges for application of PHA in the industry, nevertheless, for purposes of biotechnological process monitoring, PHA polymers should be well characterized by modern analytical strategies. Up to now, mainly the screening methods exploiting spectrofotometric detection after the Nile-red colouring are widely used, however, using this approach, no information about the polymer structure is available. Another option could be enabling of infra-red Fourier transform spectrometry (FT-IR), however this method is rather demanding for PHA isolation and extract purification. On the occasion of lack of the rapid, reliable and easy to use methods, we developed an analytical strategy exploiting the sample hydrolysis, characterization and quantitation of PHA monomers by the ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometry (U–HPLC–HRMS/MS) represented by TripleTOFTM (AB Sciex). Thanks to the high resolving power of the mass spectrometric system assuring the method selectivity, we could omit the extract purification and increase the method throughput significantly. Our method consists of several steps: *(i)* acidic butanolysis of the sample, *(ii)* extraction of butylesters by the QuEChERS approach, *(iii)* separation and detection by the U-HPLC-(ESI+)HRMS/MS method. For quantification, butylesters prepared from γ-lactones standards were exploited. As testing materials, commercially available copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and the lyophilised biomass of Pseudomonas, were examined.

Keywords: *PHA, Pseudomonas, butanolysisysis, extraction, standards, U-HPLC–HRMS/MS*

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C-2
DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR THE DETECTION OF TOXINS AND CHEMICAL CONTAMINANTS IN FOOD

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The current analytical techniques that are currently used within the food industry to detect pathogens and toxins rely on time consuming and expensive procedures such as polymerase chain reaction (PCR) and high performance liquid chromatography (HPLC). Biological and Chemical sensors offer the possibility to improve the overall analytical process by providing screening tests which will allow a higher throughput of samples in a much cheaper manner and without the need for extensive operator training. With this goal in mind we have designed a 3×3 sensor array with integrated gold working electrodes, platinum counter electrodes and silver/silver chloride reference electrodes. The chip has been characterised using cyclic voltammetry, a common electrochemical technique that studies the properties of an analyte in solution. In this case, the redox couple ferricyanide/ferrocyanide was used to compare the performance of the sensor in comparison to an ideal one electron process. Each sensor in the array was analysed to ensure uniformity across the entire chip. The influence of scan rate, cleaning procedure as well as the stability of both the reference and counter electrodes was also investigated. This chip will then be used as an electrochemical immunosensor for the detection of various mycotoxins in a range of food samples. The assay will be based on the ELISA technique and compared to this more traditional technique. Some preliminary ELISA work has already been carried out. A simple electrochemical method is also presented for the detection of the quorum sensing molecules, 4-hydroxy-2-heptylquinoline (HHQ) and 2-heptyl-3,4-dihydroxyquinoline (PQS) in food samples. Sensors for the detection of these molecules have used either complicated assays which require a number of steps or expensive materials such as boron doped diamond. We present a cheap, simple detection method using screen printed carbon electrodes. A detection limit of 4μM has been achieved by amperometry with a bare electrode. Work is currently on-going in order to try and improve this limit of detection.

Keywords: *Immunosensors, Chemical sensors, cyclic voltammetry, ELISA*

Acknowledgement: *Integrated Nanoscience Platform for Ireland (INSPIRE) and the National Access Programme (NAP).*

C-3
DEVELOPMENT AND EVALUATION OF LATEX AGGLUTINATION TESTS, IMMUNOMAGNETIC BEADS AND ELISAS FOR NON-O157 SHIGA TOXIN-PRODUCING *E. COLI* (STEC)

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Non-O157 Shiga toxin-producing *E. coli* (STEC) are responsible for gastrointestinal illnesses, as well as hemolytic uremic syndrome (HUS), the leading cause of acute renal failure in children. *E. coli* O157, the most notorious of the STECs was declared an adulterant in beef by the U.S. Department of Agriculture (USDA) in 1994. To further protect the public, the USDA has recently declared six non-O157 *E. coli* serogroups: O26, O45, O103, O111, O121, and O145 as adulterants in raw beef. Non-intact raw beef products or the components of these products found to have these pathogens are prohibited from sale to consumers. The USDA Food Safety Inspection Service (FSIS) and the Agricultural Research Service developed test protocols described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5B.03 that are now used for regulatory testing for non-O157 STEC. To comply with these testing protocols, we developed and evaluated latex agglutination tests (LAT) and immunomagnetic separation (IMS) beads for sample isolation and concentration, as well as enzyme-linked immunosorbent assays (ELISAs) for each of the six serogroups. Potential positive colonies after IMS isolation are tested with LAT to determine the STEC serogroup and are verified again with LAT after incubation on Sheep Blood Agar. The developed LAT and ELISA tests are rapid and sensitive and display a very high selectivity profile against STEC and non-STEC serogroups. Assay procedures, sensitivity, and cross-reactivity profiles against STEC and non-STEC bacterial cells will be presented.

Keywords: *E. coli, STEC, ELISA, IMS beads, Latex agglutination*

C-4
DETECTION OF STRESS HORMONE IN THE MILK USING QCM METHOD

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Animal welfare is recently focused in livestock industry. Controlling the stress level of farm animals is important not only in ethical concerns but also in high quality production. Farm animals face many stressors around the feeding environment such as temperature, feed and the number of animals per unit area. We focused a cow for an actual case. Cortisol is well known as a stress marker of the endocrine system. Some reports monitored cortisol level in cow's blood, and the concentration of cortisol was lower than 10 ng/mL. However, collecting blood is stressful for animals and controller. Non–invasive monitoring of cortisol level is desired for the daily stress test. Milk is ideal material for analyte, since the milk is collected daily operation. However, the concentration of cortisol in the milk is much lower than that in the blood, and many foreign substances are included in the milk. A sensing system for cortisol requires high sensitivity with short detection time for livestock industry. We report primitive and positive data on active stress–monitoring for livestock industry. We focus on quartz crystal microbalance (QCM) method, which is one of label–free and real time measurement of antigen–antibody interaction with simplicity, convenience and low cost. No study has been reported on cortisol detection using the QCM method. We used a NAPiCOS QCM system consisting of a thermostatic chamber and a frequency counter (Nihon Dempa Kogyo Co., Ltd., Japan). The major problem of the QCM method is reducing noise level caused by measurement environment, such as temperature and density of surrounding media. Since twin sensor removes these environmental influences, noise level can be decreased drastically. In this report, one channel was used as a reference (Ch1) and the other channel measured antigen–antibody interaction (Ch2). In addition, surface treatment is important for the detection of cortisol in the milk, since many foreign substances are included in the milk. After coating anti–cortisol on the Ch2, blocking reagents were coated on Ch1 and Ch2 to avoid nonspecific adsorption. Difference of frequency shift between the Ch2 and the Ch1 (delta) was corresponding to the amount of analyte bound on the Ch2 without environmental influence. Competitive assay was applied in this research, because the molecular weight of cortisol was too small to detect directly on QCM. We used cortisol 3–BSA as a tracer. Our sensing system was integrated with a flow injection analysis for adapting a sequential analysis in farm test. Sample volume was only 20 µL. We achieved to detect the cortisol level in the milk from 0.1 pg/mL to 400 pg/mL with quick detection time of 10 minutes including regeneration process. The detection range was appropriate for the analysis of cortisol in the milk.

Keywords: *Stress hormone, milk, QCM*

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BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

(D-1 – D-39)

D-1
CURRENT IODINE INTAKE AMONG CHILDREN AND WOMEN OF REPRODUCTIVE AGE IN KAZAKHSTAN

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Background. Iodine deficiency is a global problem representing the most common preventable cause of mental retardation. In 2003 the Republic of Kazakhstan (RK) was one of the first countries of CIS region adopted the Law on mandatory iodization of table and cattle salt and in 2010 the country was certified as reached universal salt iodization (USI). So there was the necessity to clear up current iodine situation among population of Kazakhstan.
Objectives. To assess the iodine status among children of 6–59 months and women of reproductive age (15–49) in three regions of the Kazakhstan Republic in 2012.
Methods. A population based cross sectional study was surveyed to analyze data of iodine intake among 6-59 months children (n=720) from Akmola (n=250), East Kazakhstan (n=258), South Kazakhstan (n=212) regions and non pregnant women of 15-49 reproductive age (n=701) of Akmola (n=240), East Kazakhstan (n=240), South Kazakhstan (n=221) regions. Urinary iodine excretion (UIE) was measured in casual urine samples and iodine intake was defined using ammonium persulfate digestion with spectrophoto-metric detection of the Sandell-Kolthoff reaction in resource iodine lab which successfully participated in external quality control program EQUIP (CDC, Atlanta) from 2002. The women were interviewed about salt iodization issue. Results.The median UIEs of children of Akmola, East Kazakhstan and South Kazakhstan regions were 240.3 μg/L, 207.4 μg/L and 153.5 μg/L respectively and non pregnant women of reproductive age were 258.2 μg/L, 191.9 μg/L, and 150.8 μg/L. The percentage of iodine deficient (UIE300 μg/L) children were 33.3% and non pregnant women of reproductive age were 27.9% in the three regions.The analysis of the questioners revealed 2.3% (18) of children and 3.0% (21) of women were used iodine preparations during last month.
Conclusion. In this study, iodine status in children and women of the target three regions was not in stable position. There was prevalence of iodine deficiency and excess iodine intake among children and women of the population. According to the international criteria significance level of iodine deficiency in non-pregnant women aged 15–49 years in Kazakhstan in 2012, exceeding the 20%-ing point of reference is classified as a moderate risk to public health. It indicates the necessity for periodic biological monitoring and continuing communication activity with population on long-term basis.

Keywords: *Iodine intake, children, women of reproductive age, urinary excretion*

D-2
ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY AS A TOOL FOR RAPID AND RELIABLE DETERMINATION OF FREE AMINOACIDS IN FOOD MATRICES

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Analysis of amino acids composition and their quantification is very important in terms of the assessment of nutritional values of food products, control of nutritional claims declared by dietary supplements producers. Up to now, high-performance liquid chromatography (HPLC) coupled with conventional detectors (UV, FLD), which includes the pre-/post-column derivatization of analytes, have been the most widely used technique. Nevertheless, nowadays, using of time-saving methods omitting the derivatization steps based on ultra-high performance liquid chromatography coupled with mass spectrometric systems continues to increase. Our study describes a rapid and sensitive analytical method for determination of free amino acids in food and foodstuff matrices. Because of the considerable polarity of amino acids, hydrophilic interaction chromatography (HILIC) was chosen for their effective separation. 22 underivatized amino acids were separated on Atlantis HILIC Silica column (100 x 3 mm; 3 μm) by gradient elution in the system of aconitrile/water. For the detection, two types of mass spectrometric instrumentations were tested *(i)* high resolution mass specktrometer with orbitrap mass analyzer (Exactive, Thermo Fisher Scientific), and *(ii)* tandem mass spectrometer with hybride quadrupole–ion trap mass analyzer (Q-trap 5500, AB Sciex). With regard to relatively high concentrations of amino acids present in food or dietary supplements, the quantification limits achievable were not the most important and decision making criterion. With regard to the full spectral information provided by the orbitrapMS syst em, the U-HPLC–orbitrapMS method was chosen as a better alternative in this study. Additionally to the target amino acid analysis, identification of their food-processing degradation products would be possible. Regarding the sample preparation, it differed in dependence of food matrix character. Liquid samples (protein hydrolyzates or beer) were just diluted into the acetonitrile (solvent compatible with the HILIC chromatography) and directly analyzed. Water-soluble dietary supplement samples were firstly diluted in water, and then diluted into the acetonitrile. Other water-insoluble matrices (e.g. vegetables, cereals, etc.) have to be extracted with the 0,2mM acetic acid. Within the U-HPLC–orbitrapMS method validation, repeatebilities of the method expressed as relative standard deviations (RSDs, %) were determined. For liquid or water soluble matrices the internal reference material of beer containing almost full spectrum of basic amino acids was used, the RSD values ranged between 3.8 and 6.2 %. For solid samples, where the extraction step had to be included, the RSDs were slightly higher, but not exceeding 9%. Recoveries of analytes were examined only in the case of solid samples, where the extraction of analytes was needed. For quality assurance of the method, sample of internal reference material (hydrolyzed collagen sample) was enabled.

Keywords: *Amino acids, hydrophilic interaction chromatography, ultra-high-performance chromatography, high resolution/tandem mass spectrometry*

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D-3
ALGINATE-HYDROXYPROPYLCELLULOSE MICROBEADS FOR RETINOL ENCAPSULATION

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The introduction of bioactive ingredients such as vitamins in a variety of food products requires the development of new and innovative approaches, due to the high sensitivity of vitamins to chemical and physical conditions present in the processing environment. Adverse effect of temperature, high pressure or oxidizing agents may reduce biological functionality and can influence on chemical degradation of bioactive components. One of the challenges of food fortification technology is the development of the appropriate formulations of fat-soluble vitamin A (retinol). The search for new carriers of retinol is extremely important because of its limited stability, insolubility in water and toxic effect of excess vitamin in the body. In this work we present the result of our studies on development of a new retinol delivery system. To increase the chemical stability of retinol and physically makes them easily and stably dispersible in water we prepared the retinol loaded liposomal formulation. Liposomes were characterized for encapsulating efficiency, vesicle size and zeta potential. In the next step liposome dispersion with incorporated retinol was successfully encapsulated in the alginate-hydroxypropylcellulose microbeads. The incorporation of active ingredients in microbeads enables them to be released in a controlled manner under physiological conditions. The properties of the obtained microbeads were carefully studied.

Keywords: *Retinol, Alginate, Hydroxypropylcellulose, Microbeads, Liposomes*

D-4
THE INFLUENCE OF DIFFERENT EXTRACTION METHODS OF ELEUTHERO (*ELEUTHEROCOCCUS SENTICOSUS /RUPR. ET MAXIM./ MAXIM.)* UNDERGROUND ORGANS ON THE CONTENT AND COMPOSITION OF OBTAINED ISOLATES

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Eleuthero (*Eleutherococcus senticosus /Rupr. et Maxim./ Maxim.*) is a shrub growing wild in Northeastern Asia. Underground organs of this plant (rhizomes with roots) are used in TCM as well as in western medicine as a raw material with adaptogenic activity. Main active compounds responsible for its stimulant and tonic effects are eleutherosides. According to European Pharmacopoeia dried raw material is standardized on the content of the sum of eleutheroside B (syringin) and eleutheroside E (liriodendrin). The aim of undertaken study was to compare the influence of extraction method and extraction medium on the content of eleutherosides B and E in obtained extracts. For the investigation fresh and air-dried underground organs of 3-years-old plants cultivated in experimental field of Department of Vegetable and Medicinal Plants, SGGW were used. Three extraction methods (under reflux, ultrasonic sonication and continuous extraction) as well as three extraction mediums (ethanol water solutions 40, 70, 96% and pure water) were applied. HPLC was used to determine the content of eleutheroside B and E in obtained extracts. The most efficient extraction method for both eleutherosides was continuous extraction in comparison with two other methods applied. The highest content of investigated eleutherosides was found in extracts obtained with 70% ethanol water solutions. There were no significant differences in the content of examined compounds in the extracts obtained from fresh and air- dried raw materials.

Keywords: *Eleutheroside B, eleutheroside E, under reflux, ultrasonic sonication, continuous extraction*

D-5
PHENOLIC COMPOUNDS IN RAW AND BOILED YELLOW COLOMBIAN DIPLOID POTATO GENOTYPES

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Potato (*Solanum tuberosum*) is the third crop in caloric input worldwide and it has a high potential to be developed as a source of secondary metabolites such as the phenolic compounds (PC) group. It is known that non-anthocyanin hydroxycinamic acid-like compounds (non-ACN–HCA–LC) is the main group of PC in yellow potato tubers. The aim of this work was to evaluate the effect of boiling in the amount of non-ACN–HCA–LC in eight yellow potato genotypes of a breeding program involving the diploid Phureja group. Potato genotypes were grown in a location called Usme (Colombia; 3,400 m.a.s.l). The tubers from each genotype were collected, randomized and separated into two sets. One set was analyzed raw. The other set was cooked by boiling in water and analyzed. Analyses were performed in triplicate. Non-ACN-HCA-LC were extracted with acidified methanol and analyzed by ultra-high performance liquid chromatography (UHPLC) coupled to a diode array detector (DAD). Iden tification and quantification of non-ACN–HCA–LC were carried out using their retention time and spectral UV-vis data as compared to standards at 320 nm.
Results were expressed as milligrams per 100 grams of dry weight (mg/100g DW). Results showed high variability inter-genotypes. Chlorogenic acid (ChA) was the main non-ACN–HCA–LC present in raw potatoes, ranging from 84.1±2.5 to 397.6±76.2 mg/100g DW, followed by crypto-ChA (from 0.6±0.2 to 185.3±65.4 mg/100g DW). Neo-ChA (from 1.4±0.4 to 3.1±0.5 mg/100g DW), caffeic acid (fro 0.2±0.0 to 3.5±2.4 mg/100g DW), and caffeoyl-putrescine (from 1.3±0.2 to 4.7±0.7 mg/100g DW) were less abundant. The boiling process increased in different levels the amount of non-ACN-HCA-LC depending on the genotype. In boiled potatoes ChA ranged from 102.3±9.9 to 841.1±35.4 mg/100g DW and crypto-ChA ranged from 177.9±5.0 to 1,309.3±176.1 mg/100g DW. Levels of neo-ChA (from 1.7±0.0 to 15.2±16.2 mg/100g DW), caffeic acid (from 1.2±0.3 to 6.8±0.2 mg/100g DW) and caffeoyl-putrescine (from 4.1±0.4 to 14.3±0.6 mg/100g DW) were also higher than those obtained in raw potatoes. Interestingly, while the crypto-ChA/ChA ratio ranged from lower than 0.1 to 1.1 in raw pot atoes, it ranged from 1.1 to 2.3 in boiled potatoes. The increase in the total amount of non-ACN–HCA–-LC suggests that the extraction with acidified methanol in raw potato does not give full recovery probably due to their reaction with proteins as suggested in previous work. The increase in the crypto-ChA/ChA ratio indicates an isomerization process induced by cooking. The genotype dependency on the increase in non-ACN–HCA–LC might be used as a guide in yellow-national potato breeding programs seeking greater nutritional spectrum.

Keywords: *Potato, phenolic compounds, chlorogenic acid, genotypes*
Acknowledgement: *This work was founded in part by Canadian agencies IDRC and FATDC by Grant Agreement, as well as the Government of Colombia through of MADR-CENIRED under Agreement No. CE-13158-110-02. The genotypes used in this work were supported by Contract No. RGE0069 from Ministerio de Ambiente y Desarrollo Sostenible of Colombia.*

D-6
DETERMINATION OF PHENOLIC COMPOUNDS IN ABOVE- AND UNDERGROUND ORGANS OF DROPWORT (FILIPENDULA VULGARIS MOENCH)

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Dropwort (*Filipendula vulgaris Moench, Rosaceae*) is a perennial naturally occurring on sunny, semi-dry, limestone meadows and neglected areas in Europe and Asia. All organs of this plant – tuberous roots, rhizomes, leaves and flowers are a rich source of phenolic compounds, especially flavan-3-ols and phenolic acids, and have been used in traditional European medicine as anti-inflammatory, antirheumatic, diuretic and diaphoretic agents. Moreover, the tuberous roots and young leaves are edible – cooked as a vegetable or eaten raw as a component of salads. The aim of this study was to find the optimum extraction conditions (method and solvent) for determination of phenolic compounds in extracts obtained from tuberous roots, rhizomes, leaves and flowers of dropwort. The plant material was harvested from the experimental field of the Department of Vegetable and Medicinal Plants, WULS – SGGW. Air-dried raw material was extracted using two periodic extraction methods – under reflux (traditional way of extraction for this raw material) and sonication-assisted solvent extraction, as well as two continuous extraction methods – in classic Soxhlet apparatus and in modified, automated Soxhlet apparatus (hot extraction). Ethanol 40% and 70% (*v/v*) as well as methanol were applied as extraction medium. Phenolic compounds were determined by HPLC. There were no significant differences in the content of examined compounds between extracts obtained with modified and classic Soxhlet, but the extraction was 12.5 times shorter (4 h) and the solvent consumption was 2.5 times lower (100 ml). Moreover, the modified Soxhlet apparatus allows for the automated evaporation of the solvent. In case of all other investigated methods, the extraction solvent must be removed with additional evaporator. Sonication-assisted solvent extraction was the shortest investigated method (1 h including solvent evaporation), but the content of phenolic compounds in obtained extracts was lower in comparison with Soxhlet extraction. The content of investigated phenolic compounds in extracts obtained with 40% ethanol and methanol was comparable.

Keywords: *Flavonols, phenolic acids, reflux, sonication-assisted extraction, Soxhlet apparatus*

D-7
HYDROXYCCINAMIC ACID-LIKE COMPOUNDS IN COLOMBIAN BOILED DIPLOID POTATO GENOTYPES

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Potato is the main source of energy intake in some places of Colombia. In Colombia, among different cooking methods, this tuber is mainly consumed in boiled preparations. Phenolic compounds are of interest due to their role in human health. Hydroxycinnamic acid-like compounds (HCA-LC) are the main phenolic compounds in quantity and diversity present in potato tubers. HCA-LC can be classified as non-anthocyanin (non-ACN)-HCA-LC and as ACN-HCA-LC. Chlorogenic acid (ChA), followed by others such as neo-ChA, crypto-ChA, caffeic acid, and caffeoyl putrescine are the main contributors in quantity to the non-ACN-HCA-LC in potato tubers. ACN-HCA-LC, also present in red-purple potato, are less abundant. The aim of this work was to quantify the aforementioned compounds in 60 boiled Colombian potato genotypes from the Phureja group. Non-ACN-HCA-LC were quantified by the external standard method when using an ultra high performance liquid chromatography coupled to a dio de array detector, with ChA as standard for ChA, neo-ChA, and crypto-ChA. Caffeic acid was used as standard for the quantification of caffeic acid and caffeoyl putrescine. A molecular weight correction factor was used for the latest. Quantity of non-ACN-HCA-LC was expressed as mg/100 g potato dry weight (DW). ACN-HCA-LC content was calculated from the total area of chromatographic profiles at 520 nm and expressed in relative units. The chromatographic method was tested for reproducibility within and between days with authentic ChA. In all genotypes ChA (ranging from 79.2±29.0 to 440.2±111.5 mg/100 g DW) was by far the most abundant non-ACN-HCA-LC, followed by crypto-ChA (from 7.3±8.2 to 101.6±14.3 mg/100 g DW), and by neo-ChA (from 3.4±1.2 to 30.1±7.8 mg/100 g DW). Caffeic acid (from 0.9±1.1 to 10.4±2.2 mg/100 g DW) and caffeoyl putrescine (from 0.9±0.7 to 12.0±6.8 mg/100 g DW) were less abundant. Intra-varietal variation was observed by the high coefficient of var iation (CV) found for ChA when the biological replicates were analysed (ranging from 4 to 75%), contrasting with the good reproducibility of the chromatographic method (CV for ChA lower than 4% within and between days). Furthermore, inter-varietal variability was observed not only by the differences in total content of non-ACN-HCA-LC, but also by the differences in quantity of each individual compound. This is, genotypes with the highest content of ChA did not have the highest content of the other non-ACN-HCA-LC. There was no correlation between red color, measured as ACN-HCA-LC relative content, and each individual or the total non-ACN-HCA-LC. Contribution of HCA-LC of the Colombian potato genotypes seem to be significant compared to other sources.

Keywords: *Potato, phenolic compounds, chlorogenic acid, genotypes*
Acknowledgement: *This work was founded in part by Canadian agencies IDRC and FATDC by Grant Agreement, as well as the Government of Colombia through of MADR-CENIRED under Agreement No. CE-13158-110-02. The genotypes used in this work were supported by Contract No. RGE0069 from Ministerio de Ambiente y Desarrollo Sostenible of Colombia.*

D-8
CAROTENES IN PROCESSED TOMATO AFTER THERMAL TREATMENT

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When preparation meals in the household/kitchen it is necessary to minimize damage on the sensory and nutritional quality while at the same time maximizing their safety. Data on the fate of some constituents in processed tomato products when exposed to additional heating is scarce. In this study, effects of such heating and storage on a commercially available double concentrated tomato purée were examined spectrophotometrically.

Our results indicate that upon heating at 100 to 135°C spectral profile of lycopene in the sample remained preserved. Slight hypsochromic shift of lycopene peak III occured at 135°C. Significant initial loss of lycopene of 20% was induced by heating for 20 min at 100°C. However, during the more severe treatments that followed the content of lycopene first leveled off and then increased. The same trend was evident for total carotenes (TC). However, the portion of lycopene within TC content dropped from 92.2±1.2 through 90.7±0.9 to 90.1±1.2% (n=6) when temperature was increased from 100 through 120 to 135 °C. Throughout the whole experimental scheme, peaks III/II ratio changed (n=3) from 72.0±0.3 at 100°C through 69.3±0.5 at 120°C to 66.4±2.3 at 135°C. This data may indicate gradual increase in *cis*-isomer(s) portion in the mixture with *trans*-isomer of lycopene in the sample during thermal treatment.

By virtue of product's appearance and spectral evidence, lycopene most likely underwent degradation and isomerization processes after thermal treatment of a double concentrated tomato purée. Data collected for processing exceeding ≥120°C provided the evidence that contribution of non-enzymatic browning reaction products antioxidant preserving action and higher extractability of carotenes from the tomato matrix should also be taken into account. Despite the favourable effects of MRP precaution is needed since during processing of tomato products at elevated temperatures there is a possibility for formation of toxic compounds.

The temperature- and light-controlled storage of double concentrated tomato purée (-18°C, dark) over a month showed that content of lycopene increases (0.2 to 9.9%) possibly due to *cis-trans* re-isomerization. On the other hand storing of hexane solutions for 2 months at the room temperature resulted in severe *trans-cis* isomerization and/or photo-oxidative degradation that increased in proportion to the severity of thermal treatment.

D-9
PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY IN THE LEAVES OF STEVIA (*STEVIA REBAUDIANA*) INTRODUCED INTO GEORGIA

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In recent years plant materials rich in phenolic compounds are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Phenolic compounds are plant secondary metabolites widely spread in nature. They are commonly found in both edible and inedible plants. Phenolic compounds possess unique functions and nutritional values including antioxidant, antimicrobial, antimutagenic and antitumor activities. Thanks to their antioxidant properties, these compounds can protect against degenerative diseases (i.e. heart disease and cancer) in which reactive oxygen species, such as superoxide anion, hydroxyl and peroxy radicals are involved. Phenolic compounds represent the most desirable phytochemicals due to their valuable properties and potential use as additives in food industry, cosmetics, medicine. In this point of view the investigation of plant raw material in order to reveal rich sources of phenolic compounds is of high importance. The aim of the present study was to determine the content of total phenolic compounds as well as antioxidant activity in the leaves of stevia (*Stevia rebaudiana*) introduced into Georgia in order to use them for production of biologically active natural food additive for functional food. The content of phenolic compounds in the leaves of stevia (*Stevia rebaudiana*) introduced into Georgia was determined by the Folin-Ciocalteu method. The total antioxidant capacity was estimated by the ferric reducing antioxidant power (FRAP). The stevia leaves were found to contain a large amount of phenolic compounds (12.2% of dry matter). The water extract of stevia leaves revealed high antioxidant activity (8.0 [Fe^{2+}] mmol.l-1 on 1 g/l concentration of the extracts) and showed only about 1.8 and 2.4 times less antioxidant potential than Georgian rolled green tea and instant green tea extract (respectively) which are characterized by high antioxidant activity. Due to the high content of phenolic compounds the leaves of stevia introduced into Georgia may be used successfully for production of biologically active food additives.

Keywords: *Stevia leaves, antioxidant activity, phenolic compounds*

Acknowledgement: *The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7) under grant agreement # 293514.*

D-10
INULIN CONTENT IN DRY EXTRACTS OF TOPINAMBUR (*HELIANTHUS TUBEROSUS* L.) OBTAINED BY DIFFERENT DRYING METHODS

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Inulin is a soluble naturally occurring oligosaccharide belonging to a group of carbohydrates known as fructans. It has a number of health benefits. It helps by diabetes, arteriosclerosis, obesity and hearth-vascular diseases and used as a prebiotic agent in functional foods to stimulate the growth of beneficial intestinal bacteria. Due to the soluble in hot water it easily incorporated into drinks, dairy products, and baked goods. Inulin has a lot of characteristics beneficial to functional foods. It can improve taste, texture, and moisture in many foods. As a fructan, inulin can be substituted for sugar when a reduced sugar content is desirable. Nowadays, all over the world, a lot of attention is paid to replacing artificial food additives with natural biologically active ones, which will intensify technological processes and at the same time give the final product some kind of functional properties. In this point of view the study of inulin-contain ing plants is very important. Inulin is naturally present in many different foods. One of the richest sources of inulin is topinabur (*Helianthus tuberosus L.*) tubers. The aim of the present work was to determine content of inulin and soluble sugars in the extracts of tubers of topinambur (introduced into Georgia) obtained by freeze drying and spray drying methods in order to produce a multifunctional biologically active food additive. Extraction of topinambur tubers was carried out at 80°C. Obtained extract with content of dry matter of 11. 5% was dried by two different drying methods - freeze –drying and spray drying methods. Content of inulin and soluble sugars was determined spectrophotometrically, according to the method of Beliakov and Popov (1998). The inulin content in the freeze – dried extract of topinambur tubers was found to be 20–22%, which is about 2 times higher than the inulin content in the spray-dried extract of topinambur tubers. As for the content of soluble sugars, it was much higher in spray - dried extracts (13–15 %) than in the freeze–dried extract (4–5%) of the tubers of this plant. The higher amount of soluble sugars in spray-dried extracts may be caused by decomposition of inulin at high temperatures peculiar to the spray drying method. Thus, the method of drying significantly affects the content of inulin and soluble sugars in the dry extracts of topinambur tubers.

Keywords: *Inulin, topinambur tubers, functional food*

Acknowledgement: *The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7) under grant agreement # 293514.*

D-11
STABILITY OF BIOLOGICALLY ACTIVE NATURAL FOOD RED COLORANT FROM POKEBERRY (*PHYTOLACCA AMERICANA* L.) FRUITS

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Nowadays, consumers increasingly prefer food products which are fully natural. This has driven an increase in the use of food additives derived from biological sources. Of particular interest are natural colorants. Natural colorants not only prevent the health hazards posed by synthetic colorants but also confer various health benefits to the diet. In addition to the pigments, natural colorants may contain valuable biologically active compounds, such as amino acids, phenolic compounds, carbohydrates as well as trace elements, improving the quality of the final product. Therefore, intensive research has been carried out all over the world to identify plant raw materials and develop technology for production of natural food colorants from them. Recently the technology of production of a natural red food colorant from pokeberry (*Phytolacca Americana* L.) fruits has been worked out at Durmishidze Institute of Biochemistry and Biotechnology of Agricultural Uni versity of Georgia (Tbilisi, Georgia). The colorant was shown to be rich in biologically active compounds, trace elements such as iron, zinc, and manganese as well as red coloring substances. It was found to be characterized by antioxidant, antimicrobial and antiradiation activities. The aim of this study was to determine pH, temperature and storage stability of a biologically active food red colorant from pokeberry fruits and investigate its coloring potential. The colorant was prepared by the following procedures: fresh juice of pokeberry fruits was boiled twice for the purpose of detoxification. The green tea dry extract was gradually added to the hot juice. The obtained mixture was filtered and spray-dried. The dry colorant from pokeberry fruits revealed high storage stability. It was shown to be quite stable at high temperatures (90–100°C) and in the pH 5–6 range. Natural red colorant from pokeberry was effectively used to color different kinds of food products with pH ranging from 3 to 7. The colorant did not change organoleptic properties of the colored food products when used at concentrations between 0.1–0.25%. The biologically active natural red colorant from pokeberry fruits with high stability and coloring potential may be used successfully in food industry to color a wide range of food products.

Keywords: *Pokeberry fruits, green tea extract, antioxidant, natural red colorant*

Acknowledgement: *The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7) under grant agreement # 293514.*

D-12
HPLC METHOD FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF CAROTENOIDS IN CARROT

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The aim of the study – to determine optimal parameters for extraction, identification and quantification of carotenoids in carrot by high performance liquid chromatography. The objects and methods of researches.
The object of research were samples of carrot 2013 year's harvest. Acetone and n-hexane were used for the extraction of carotenoids. Solvents were evaporated to dryness in a rotary evaporator under vacuum and under a stream of nitrogen. Pigments were dissolved in various solvents and were transferred to vials. The HPLC method was used for analysis of carotenoids. Chromatographic separation was performed on Agilent 1200 series liquid chromatographic system which equipped with diode array detector and column ZORBAX Eclipse Plus C18 (3.0×100 mm; 1.8 μm). Temperature in autosampler was +10°C, injection volume – 10 μl, temperature of column – +22°C, flow rate – 0.4 ml/min. The mobile phase was a mixture acetonitrile – methanol – ethyl acetate (73:20:7, v/v/v). Results were monitored at 440, 450 and 480 nm. β-carotene (Fluka, №22040) and lutein (ROTH, №5671) were used as standards .
The results of research. Optimal sample of carrot for analysis of carotenoid was determined as 5 g. A complete extraction of pigments from preground carrot was obtained with 10-multiple volume of acetone (50 ml). After extraction and evaporation the residue was reconstituted with 1,5 ml of acetone and was transferred in a screw-cap vial for HPLC analysis. It has been found experimentally that acetone was the best solvent for carotenoids. Using of mixture acetonitrile – methanol – ethyl acetate (73:20:7, *v/v/v*) as mobile phase in proposed chromatographic conditions gave a good separation of the main carrot carotenoids: lutein (RT~2.8 min), α-carotene (RT~28.0 min), β-carotene (RT~30,0 min). The calibration curves have a linear character with correlation coefficient R = 0.99 in concentration ranges: 0.001 – 0.050 mg/ml for lutein and 0.1 – 1.0 mg/ml for β-carotene.
Conclusion. The analytical results revealed that suggested method is fast, relatively simple and suitable for determination of the main carotenoids of carrot: α-, β-carotene, lutein. Further research is necessary to optimize HPLC method for separation and identification of other carotenoids in extracts of carrot.

Keywords: *Carrot, carotenoids, separation, identification, HPLC*

D-13
QUERCETIN AND KAEMPFEROL MALONYLGLUCOSIDES IN BLACK CURRANT LEAVES

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Extractions from black currant leaves are traditionally used in Europe for the treatment of rheumatic diseases [1]. Their anti-inflammatory and diuretic properties seem to constitute the basis of this treatment. Owing to literature data it could be assumed that the polyphenols in their conjugated forms are responsible for these effects. The main polyphenolic components in black currant leaves are quercetin and kaempferol malonylglucosides. To study their occurrence, the polyphenolics in the leaves of various black currant cultivars were analyzed by HPLC–DAD and HPLC–MS. Furthermore, the changes caused by different drying procedures and technological processes, e.g. hot-water extraction and spray-drying, were also examined. The investigations revealed that the malonylglucosides of quercetin and kaempferol are unstable; the extent of the hydrolysis depends on the respective procedures.

[1] The European Scientific Coorperative on Phytotherapy (ESCOP): Monograph, Thieme, 2003, 2. Edition, http://escop.com/publications

Keywords: *Polyphenols, black currant leaves, quercetin malonylglucosides*

D-14
ANALYSIS OF VITAMIN D IN FEED BY UPLC–MS/MS

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Various forms of vitamin D, namely cholecalciferol (vitamin D3), ergocalciferol (vitamin D2) and 25-hydroxy-cholecalciferol, are included in the European Union Register of Feed Additives. Maximum limits for these compounds have been established and vary depending on the feed. The simultaneous use of different forms of vitamin D is prohibited or strictly directed [1, 2]. Central Institute for Supervising and Testing in Agriculture inspects the animal feedstuffs, premixtures and mineral feeds for various parameters including the content of cholecalciferol. Method for determination of vitamin D based on BS EN 12821:2009 [3] has been validated. Sample preparation is based on liquid-liquid extraction after alkaline saponification and determination is carried out by ultra-performance liquid chromatography coupled to tandem mass spectrometry using positive electrospray ionization (UPLC–MS/MS). Deuterated cholecalciferol is used as internal standard for the quantification of cholecalciferol in complex matrices. Since 2012 almost 180 feed samples have been inspected using this method. Feed materials with non-complying contents of Vitamin D were found rarely. The method originally developed for the determination of cholecalciferol was subsequently extended to other fat soluble vitamins and related compounds, such as vitamin A, vitamin E, ergocalciferol, 25-hydroxycholekalciferol, 7-dehydro-cholesterol and dihydrotachysterol. Some of these compounds may naturally occur in feed materials and thus may contribute to the total amounts of vitamins in the artificially enriched feeds. Presence of these compounds in feeds and raw materials for feed production has been investigated in order to find out whether the vitamin levels were complying with the declaration values or/and the legislation limits.

[1] Council Directive 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs.
[2] Commission regulation (EC) No 887/2009 of 25 September 2009 concerning the authorisation of a stabilised form of 25-hydroxycholecalciferol as a feed additive for chickens for fattening, turkeys for fattening, other poultry and pigs.
[3] BS EN 12821:2009. Foodstuffs. Determination of vitamin D by high performance liquid chromatography. Measurement of cholecalciferol (D3) or ergocalciferol (D2).

Keywords: *Vitamin, feed, UPLC–MS/MS*

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D-15
OXIDATION OF PHENOLIC COMPOUNDS IN PLANTS AND PLANT FOODS

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Most phenolic compounds can be easily oxidized, either enzymatically or non-enzymatically, and are unstable in oxidizing environment. Surprisingly, only a few oxidation products are known to date. These substances mainly include polymers, such as melanin and lignin, and some specific low-molecular weight molecules that include lignans and oligomeric proanthocyanindins. By using both, enzymatic and non-enzymatic in vitro approaches, we found that the most common oxo-products are various dimers, such as diferulic, disinapic and dicoumaric acids, which are relatively stable. These compounds were then structurally elucidated by NMR and monitored by specific UPLC–MS/MS methods in several plants and plant foods. As a result, we were able to confirm that these substances are naturally present in plants/foods as free compounds. We are currently testing safety and general biological activity of these oxidation products.

Keywords: *Phenolics, oxidation, mass spectrometry*

Acknowledgement: *This study was supported by project GACR 503/12/P166*

D-16
METHODS FOR EVALUATION OF ANTIOXIDANT ACTIVITY OF HERBS

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Antioxidants are compounds which are important for oxidative stability of fats and oils particularly with higher content of unsaturated fatty acids. Antioxidant activity of herbs can be evaluated by screening methods which possess information about total activity of antioxidant. Because of their different reaction mechanisms, it is not possible to apply only one method to be able to describe antioxidant activity of different types of antioxidants. These methods are based on the evaluation of ability to scavenge free radicals (e.g. DPPH method) or reducing activity (e.g. FRAP method). Oregano, mint and lemon balm showed very good antioxidant activity determined by DPPH method. On the other hand, antioxidant activity of winter savory, sage, marjoram or rosemary determined by the same method was moderate or poor. Results of three different screening methods (DPPH, total phenol compounds and FRAP) showed strong correlation between antioxidant activity determined by DPPH method and the content of total phenolics ($R > 0.95$ for 34 samples) and between antioxidant activity determined by DPPH and FRAP method in water extracts of selected herbs ($R > 0.88$ for 7 samples). Classical Schaal test (in normal arrangement or in thin layer of fat), any method of accelerated oxidation, e.g. Oxipres or Rancimat tests and/or any method for determination of any oxidation products, e.g. content of polymeric triacylglycerols, determination of oxidizing fat by LC etc. can be used for determination of effectiveness of antioxidants in fats and oils during their frying or storage Oxidative stability of fats and oils and effectiveness of antioxidants depend on many external conditions, e.g. temperature, access of oxygen and light or presence of metals. Effectiveness of antioxidants decreases with higher temperature and partial pressure of oxygen. This can be seen from the results of high oleic rapeseed oil oxidation with and without rosemary extract addition monitored by Schaal and Oxipres methods. Effectiveness of this extract was poor when the Oxipres test was used in comparison with the Schaal test. Very good ability to inhibit pork lard oxidation was found in case of rosemary, winter savory, sage, Greek oregano, or marjoram and their extracts. The results of screening methods and methods for monitoring antioxidant activity of fats and oils do not exhibit positive correlation.

Keywords: *Antioxidant activity, herbs, screening methods, Schaal test*

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D-17
ANTIOXIDANT PEPTIDE DERIVED FROM OVOMUCIN HYDROYSATE OBTAINED AFTER MICOBIAL PROTEASE TREATMENT

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Among the various bioactive peptides derived from food proteins, those having antioxidant properties are important because free radicals produced by oxidative reactions might result in different disorders such as diabetes mellitus, arthrosclerosis, cancer, and coronary heart disease, in addition to causing allergies and aging20−22 due to damage to cellular components. Antioxidant peptides have been identified from hen egg white lysozyme hydrolysate by hydrolysis with papain, trypsin, and a combination of these two enzymes as well as from ovotransferrin hydrolysate following enzyme treatment. Thus far, antioxidant activity has not been reported in the case of ovomucin or its hydrolysate. Ovomucin hydrolysis was carried out using microbial protease according to different incubation times. These ovomucin hydrolysates exhibited 85% antioxidant activity as measured by the 2,2′-azinobis(3-ethylbenzo-thiazoline-6-sulfonic acid) assay after a 2 h incubation with protease and retained 90% activity until 24 h. At an incubation time of 4 h, the activity of ovomucin hydrolysates reached approximately 90%, corresponding to 115 μM gallic acid equivalent, regardless of the proteases used. The partially purified fraction of the hydrolysate by ultrafiltration and reverse-phase high-performance liquid chromatography was collected and then analyzed by liquid chromatography electrospray ionization mass spectrometry. Two peptides, LDEPDPL and NIQTDDFRT, in this fraction were identified. The antioxidant activities of these two synthesized peptides were measured to be 51.8 and 24.7% by the 2,2- diphenyl-1-picrylhydrazyl assay. The peptide in this study, LDEPDPL, contains two nonpolar amino acids, Pro. This indicates the peptide containing nonpolar amino acid residues along with hydrophobic residues could be effective for antioxidant activity. Further investigation into the antioxidant activity of such peptides is an attractive line of research for the potential application of such peptides in the food industry as antioxidant agents or food additives.

Keywords: *Antioxidant peptide, ovomucin hydrolysate*

D-18
(-)-EPICATECHIN METABOLISM IN HUMANS; FROM FOOD COMPONENT TO HIS BIOEFFICACY

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A large number of epidemiological studies have established an inverse relationship between chronic consumption of flavanol rich foods and the risk of cardiovascular diseases. These epidemiological observations are supported by numerous nutritional intervention studies using flavanol-rich foods and even purified flavanols. Due to its high concentration in human diets (-)-epicatechin is one of the most studied flavanols. Following absorption in the gastrointestinal tract, (-)-epicatechin is completely metabolized. These metabolites are chemically different from the aglycone forms found in foods, are the compounds that reach the circulatory system and other target organs. Therefore, it is important to identify and quantify these circulating metabolites compounds in order to investigate which is/are the bioactive metabolite(s). With this objective, we aim to reveal the (-)-epicatechin complete metabolite profile. Initially, we developed and validated an analytic al methodology to quantify the metabolites in biological matrices using synthetic (-)-epicatechin sulfates, glucuronides, and methyl-sulfate/glucuronide standards. Then, we evaluated the complete (-)-epicatechin metabolism after administration of flavanol-rich foods in humans. Afterwards, these relevant metabolites were tested in some in-vitro systems to evaluate their bioefficacy.

Keywords: *(−)-Epicatechin, Cocoa, Chocolate, Metabolites, Humans*

D-19
NOVEL, UNIVERSAL APPROACH FOR THE MEASUREMENT OF NATURAL PRODUCTS IN A VARIETY OF BOTANICALS AND SUPPLEMENTS, PART 2

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Botanicals contain a great diversity of compounds that exhibit wide variation in their physicochemical properties. Although no single analytical method is available to measure all potentially active components, HPLC with charged aerosol detection Is a nearly universal approach that nonselectively measures any nonvolatile and many semivolatile compounds; that is, charged aerosol detection does not require that analytes be ionizable (as required for mass spectrometry) or contain a chromophore (as required for UV spectrophotometry). A number of isocratic and gradient HPLC/UHPLC methods with charged aerosol detection were developed and evaluated for the measurement of phytochemicals extracted from a variety of botanicals including: steroidal and pregnane glycosides from Hoodia gordonii; steroidal lactones from Withania somnifera; flavonolignans from milk thistle (Silybum marianum); triterpene glycosides from black cohosh (Cimicifuga racemosa); and ginsenosides from ginseng (Panax ginseng). Analytes showed consistent response independent of chemical structure (typically < 10% variability between compounds corrected for gradient elution). All methods had a wide dynamic range (~four orders of magnitude), good sensitivity (typically low ng levels of detection), and excellent reproducibility (RSDs typically < 2%) even at low detection levels. Comparative data from ELSD and UV detection will also be discussed. The charged aerosol detector is a sensitive, mass-based detector, especially well-suited for the determination of any nonvolatile analyte independent of chemical characteristics. The detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles, which become charged in the mixing chamber. The charge is then measured by a highly sensitive electrometer, providing reproducible, nanogram-level sensitivity. This technology has greater sensitivity and precision than ELSD and RI, and it is simpler to operate than a mass spectrometer (MS).

Keywords: *Botanicals, HPLC, charged aerosol detection*

D-20
ANTIOXIDANT CAPACITY AND CHEMICAL ANALYSIS OF AÇAÍ (*EUTERPE OLERACEA MART.*) FRUIT FRACTIONS

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Açaí is an exotic berry from Brazilian Amazon and is at the top of the super food list. These fruits show attractive color characteristics and beneficial properties, such as antioxidant activity due to the high content of anthocyanins. It is known that the diary consumption of these super fruits can contribute to improve the serum antioxidant status. On other hand, the knowledge of the bioactive and chemical compounds present in seed, pulp and peel of these fruits could contribute to a competitive agribusiness. From this point of view, a new açaí native fruit of floodplain of the Brazilian state of Pará was fractionated in peel, pulp, peel plus pulp, and seed, using a new technology for singly evaluation of the antioxidant capacity, bioactive substance contents and composition. Higher lipid content (13.2%) was present in the açaí pulp fraction and gas chromatography (GC-FID) analysis showed unsaturated fatty acids (71.8%). Peel and seed fractions had the highest dietary fiber content (86.05 and 83.38%, respectively), being insoluble dietary fiber predominant in the peel. Peel, which yield was of 2% in the process, has the higher total anthocyanin (372.8 mg/100g) and total phenolic compounds (2584 mg/100g) levels and also the high antioxidant capacity (45.8 µmol TE/g). These results show promising perspectives for the exploitation of açaí fractions to new tropical products with considerable levels of nutrients and antioxidant capacity.

Keywords: *Antoxidant capacity, Euterpe oleracea, fruit fractions, GC-FID*

Acknowledgement: *Fapesp*

D-21
FAST AND ACCURATE SECOND ORDER FLUORESCENCE-CHEMOMETRIC PROCEDURE TO QUANTIFY THE PRINCIPAL FLAVAN-3-OLS CATABOLITES AFTER THE CONSUMPTION OF VEGETAL FOODSTUFF

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Polyphenols, compounds highly abundant in vegetal foodstuff, have attracted great scientific interests, mostly due to the numerous health effects that have been attributed to them. Among the polyphenols, flavan-3-ols represent a relevant group for being the most abundant in the diet. The analysis and quantification of the flavan-3-ol catabolites resulting from the gut microbiota could report valuable information to assess their absorption mechanisms and bioavailability, besides to provide information of the correlation between intake and potential health effects. Thus, the main objective of this work was to establish alternative analytical methods based on the application of second chemometric procedures (PARAFAC and SWALTD) applied to luminescent signals in order to achieve, rapidly and reliably, the concentration of the main catabolites of flavan-3-ols in urine. The luminescent properties of the main flavan-3-ols catabolites have been studied with the aim of establishing the most appropriate conditions in terms of sensitivity and stability. Once the experimental conditions were optimized, an experimental design procedure was carried out for system modeling. The proposed procedure was successfully applied to quantify the concentration of the selected flavan-3-ols catabolites in urine samples after enriched foodstuff consumption.

Keywords: *Flavan-3-ols; catabolites; chemometrics; luminescent*

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D-22
EVOLUTION AND HPLC ANALYSIS OF TRANS-RESVERATROL IN ROMANIAN GRAPE BERRIES SKINS AND WINES

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Trans-resveratrol (3,5,4'–trihydroxy-trans-stilbene), a stilbene naturally present in a large number of plant families including *Vitis vinifera* L., is a powerful antioxidant found in grape skins, grape seed and wine. Today there is a revived interest on wine consumption as medical treatment for various disorders, due to the similar therapeutic effects of resveratrol in wine to those for isolated resveratrol. To obtain information on the production of trans-resveratrol during the grape ripening, berry skins selected from five red grape varieties (*Vitis vinifera* L.) – Pinot Noir, Merlot, Cabernet Sauvignon, Mamaia and Feteasca Neagra from Murfatlar vineyard (Romania), were sampled and analysed weekly, from August to September. The study focused on the extraction procedure, including solvent type, extracting method, temperature and duration, followed by HPLC determination of resveratrol in grape skins and produced wines. Adequate extraction was attained with methanol and ethyl acetate treatment followed by quantification by high-performance liquid chromatography coupled to a ultraviolet-visible diode array detector (HPLC–DAD). The highest trans-resveratrol content was shown in the red varieties, Pinot Noir and Feteasca Neagra, both in berry skins and wines. An increase in trans-resveratrol content was observed during the first ripening stage, followed by a decrease near maturation in all grape varieties and it can be concluded that a higher degree of grape ripeness leads to a lower trans-resveratrol content.

Keywords: *Grape, HPLC, trans-resveratrol, wine*

Acknowledgement: *This study has been financed by the Romanian Ministry of Education and Research, National Authority for Scientific Research, 19N/2009 NUCLEU Program, under Project PN 09190204: „Researches regarding some vegetal anthocyanic and anthocyanidic compounds in order to use them as food natural colours"–Project Responsible PhD Violeta Niculescu*

D-23
SIMULTANEOUS ANALYSIS OF VITAMIN B12, FOLIC ACID AND BIOTIN IN A VARIETY OF SAMPLES USING IMMUNOAFFINITY COLUMN CLEAN-UP PRIOR TO UV–HPLC

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Vitamins are organic molecules that are necessary for normal metabolism and are either not synthesized in the body or are not synthesized in adequate quantities. Consequently, vitamins must be obtained from the diet. Most vitamins function as coenzymes or cofactors and there are various deficiencies related to different vitamins. R-Biopharm has developed a multi-vitamin immunoaffinity column that analyses vitamin B12, folic acid and biotin with one extraction and HPLC run. The column offers improved clean-up and concentration of the vitamins from the sample giving much cleaner chromatograms and therefore providing more accurate and sensitive detection. The procedure is based on monoclonal antibody technology which makes the test highly specific, sensitive, rapid and simple to perform. The vitamins are extracted from the sample according to the recommended extraction procedure. The extract is then diluted with buffer, filtered and passed through the immunoaffinity column where binding takes place between the antibody and the vitamins. The column is washed to remove any unbound material and the vitamin is then released by the antibody following elution before injection onto HPLC.

Keywords: *B12, folic acid, HPLC*

D-24
SIMULTANEOUS DETERMINATION OF WATER-SOLUBLE VITAMINS IN BABY FOOD BY LC–ESI–MS/MS

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Vitamins are essential to human health, and dietary supplements containing vitamins are widely used by individuals hoping to ensure adequate intake of these important nutrients. There is a need for analytical methodologies for the simultaneous determination of various vitamins, which would contribute significantly in assessing dietary intake of vitamins from all sources, including foods, dietary supplements, and fortified foods. Vitamins naturally present in foods may occur in different chemical forms, with levels ranging over several orders of magnitude. The simultaneous determination of water-soluble vitamins in baby food is a challenging task considering the complexity of the matrix and differences in vitamins' levels. In this study, a LC-MS/MS method was developed and validated for the simultaneous determination of ten water soluble vitamins of significant biological importance, applied in baby food: thiamine (B1), riboflavin (B2), niacin (B3), nicotinamide (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9), cyanocobalamin (B12) and ascorbic acid (vitamin C). The sample preparation involves a simple extraction with ammonium acetate (pH 4.5) followed by deproteinization with trichloroacetic acid. The separation was accomplished on a X-Select HSS T3 (Waters, 100 mm × 2.1 mm, 2.5 μm) RP analytical column and the mobile phase consisted of methanol and ammonium acetate at pH 4.5 (for vitamins C, niacin, B1, B6 elution) and 3.6 (for nicotinamide, B5, B9, B12, B7, and B2 elution). The chromatogram is divided into two segments for negative/positive polarity switching and positive ESI, respectively. The method is "fit for purpose", with method limits of detection ranging from 0.015 μg/g (vitamin B12) to 252 μg/g (vitamin C), consistent to the expected concentrations in the baby food samples. The inter-day precision (%RSD) was ranged from 0.52% (vit. B3) to 6.2% (vit. B9), and the recoveries were ranged between 66% (B12) and 115% (B5).

Keywords: *Vitamins, LC–ESI–MS/MS, baby food*

D-25
QUALITY ANALYSIS OF WHOLE AND CRUSHED COFFEE BEANS BY DIFFERENT ROASTING METHODS

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The aim of this work to qualify the difference of aroma and Antioxidant capacities between whole coffee beans (WCBs) and crushed coffee beans (CCBs) used as a control, experimental group respectively. Roasting degree was equalized by trigonelline content and chromaticity of L value. The chromaticity of L value of WCBs and CCBs were, not significantly different, 19.50±0.43, 19.02±0.75 respectively. In antioxidant capacities of WCBs and CCBs were slightly different, the total phenolic compounds by Folin-Ciocalteau assay of WCBs and WCBs were 40.53±1.23, 43.47±0.54 respectively. In volatile analysis, WCBs had a variety types of VOCs than CCBs. For example, VOCs of WCBs and CCBs 26 and 20 respectively. In this experimental CCBs' roasting reduce more than 20% of roasting energy in comparison with commercial roasting (WCBs' roasting) energy. In detail, WCBs' and CCBs' energy consumption were 1.4wh/g and 1.1wh/g respectively. As a result, there are no s ignificant difference between WCBs and CCBs.

Keywords: *coffee bean, energy, roasting, antioxidant capacites, volatile compounds*

Acknowledgement: *This experiment was supported by Small and medium business administration.*

D-26
FLUORESCENCE SPECTROSCOPY IMAGING AS A METHOD FOR TOTAL ANTIOXIDANT CAPACITY EVALUATION

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These days, antioxidants have received much attention as food components. They help to deactivate free radicals found in human body. Since food is the main source of antioxidants it is important to include into every day diet food products with increased level of antioxidants. The antioxidant richness of food product can be evaluated using different types of assays. Mainly, they are based on a certain chemical reaction between artificially generated free radicals and antioxidants contained in a food sample [1]. The reaction is monitored using a suitable instrumental technique, e.g. spectrophotometry [2] or fluorimetry [3]. However, these methods are time and reagent consuming. Moreover, they require either incubation of samples at a particular temperature or measurements carried out over a long period of time. Many antioxidant compounds present in food products exhibit fluorescent properties. We focus on the development of a method facilitating evaluation of total antioxidant capacity of food samples on the basis of their fluorescence images and extended with chemometric modeling.

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Keywords: *Chemometrics, Atioxidants*

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D-27
CHARACTERIZATION OF ANTIOXIDANT COMPOUNDS IN WASTE FRUIT FIBERS

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Nowadays, consumers have an increasing interest in maintaining a healthy diet and their lifestyle. With this in mind, food industries pay attention to several natural by-products from agrofruit industrial transformations [1]. These by-products, obtained from processed fruit and vegetables, arise as a novel and economic source of healthy functional ingredients [2]. In this study, we focused on the major functional antioxidant compounds of fruit juice industry by-products, such as polyphenols, carotenoids and chlorophylls. Each family of compounds has been evaluated in residues obtained from various processed fruits and vegetables (e.g apple, peach, pear, orange, tangerine, lemon and carrot) after juice extraction. Polyphenols, carotenoids and chlorophylls were quantified in order to obtain a characteristic profile and evaluate the changes observed between the obtained fruit waste and those related in the bibliography for the fresh fruit. To quantify the antioxidant compound profiles, the raw material was extracted with a process depending on the group of compounds and followed by an UHPLC–PDA analysis. Moreover, mass spectrometry was used for the identification of these compounds without commercially available standard. Identification was done using the combination of full scan and MRM modes together with bibliography information [3] in the case of phenolic and carotenoid compounds. In general the main polyphenols were the same in the wastes than in fresh fruit. In apples and drupe fruits, the profiles were dominated by chlorogenic acid and flavonols, while in citrus family flavanones were the most abundant. The same results were observed with the profile of carotenes. Wastes with highest amounts were orange and tangerine. Meanwhile, carrot waste showed a large amount of beta-carotene.

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Keywords: *Fruit juice wastes, polyphenols, carotenoids, UHPLC*

D-28
ANALYSIS OF OXYSTEROLS IN FOOD

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Study of sterols oxidation and the consequences of these reactions are mainly focused on the oxidation of cholesterol because its oxidation products are generally considered as undesirable in terms of health. Many studies have shown that cholesterol oxidation products are proven cytotoxic and mutagenic and exhibit significant inflammatory effect. Their contribution to the development of chronic diseases such as atherosclerosis and related cardiovascular diseases, some types of of cancer and neurodegenerative diseases is also important. Some authors consider oxidation products of phytosterols as harmless, because they are hardly absorbed in the digestive tract. Therefore, oxidation of phytosterols is still monitored only marginally. But these substances have a similar structure as cholesterol oxidation products and we can assume that they can participate in the development of certain colon diseases (including cancer, increases intestinal permeability, inflammation, etc.). Formation of sterols oxidation products (SOPs) in food rich in sterols is usually initiated by high temperature and the presence of oxygen. Mainly 7-oxo, 7-hydroxy and 25-hydroxy derivatives of phytosterols and cholesterol and certain types of epoxides are formed during the oxidation of sterols. The first step in the analysis of sterols (after an eventual extraction of the fat fraction) is their separation from triacylglycerols. For this purpose, the extraction of the unsaponifiable fraction with organic solvent is the most common method. Saponification may be carried out at room temperature (approximately at 25°C), but this procedure is too long (20 hours) and its effectiveness is poor. Saponification at elevated temperature is another possibility. But, this process may lead to secondary oxidation and polymerisation of some oxysterols and other oxidation changes of sterols. For example, it was shown that the relatively rapid oxidation of 7-hydroxycholesterol to 7-oxocholesterol occurs at these temperatures commonly used for saponification. Separation of sterols and their oxidation products using a solid phase extraction (SPE) can solve this problem. Gas chromatography with MS detection can be than used for the analysis of sterols. From previous studies it is clear that SOPs can have a negative impact on human health. Actual daily intake of SOPs is relatively low, but it is well known that even chronic intake below the threshold levels of certain xenobiotics (particularly lipophilic) can significantly affect the health. Most research of sterols oxidation products is focused on different model systems. This work is focused on the optimization of SPE methods of separation and analysis of sterols and oxysterols in real food of animal origin and in vegetable oils.

Keywords: *Sterol, oxysterols, SPE, food*

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D-29
ANALYSIS OF PHYTOSTEROL FATTY ACID ESTERS IN FOOD MATRICES WITH LC–MS/MS: PROSPECTS AND LIMITATIONS

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The interest in phytosterols and their fatty acid esters is generally due to their potential in decreasing plasma levels of low density lipoprotein (LDL) cholesterol, which is a potential risk factor for cardiovascular diseases. Up to date, only few methods are able to analyze individual, intact phytosterol fatty acid esters. Consequently, a LC–MS/MS method has been developed, which is able to resolve various single esters with chromatographic separation on a C4-RP–HPLC column (Pro C4, 150 × 3mm i.d., 5µm particle size, YMC Europe, Dinslaken, Germany). Gentle ESI ionization mode does not induce in-source fragmentation, and specific triple quadrupole analysis proved to be a very sensitive tool using the detection of ammonia adducts and the mass-transitions caused by the loss of the fatty acid. This method has been thoroughly validated in different food matrices. Limitations and inaccuracies, like double mass transitions because of the similar structures and molecular weights, interfering stanol esters, and matrix effects have been evaluated. Cholesterol esters were used as internal standards and phytosterol fatty acid esters of ß-sitosterol, campesterol, stigmasterol and brassicasterol have been synthesized separately for method validation. Low determination limits (0.3–1.8 µg/g in cereals and 0.4–3.0 µg/g in vegetable oil, depending on the fatty acid) and recovery values of 77–115% proved the suitability and sensitivity of the method. To the best of our knowledge, a stable isotope dilution assay (SIDA) for analyzing phytosterol fatty acid esters is lacking. Therefore a comparison between (a) cholesterol linoleate, (b) sitosterol $^{13}C_{18}$ linoleate and (c) stigmasterol $^{13}C_{18}$ linoleate as internal standards has been performed and no advantage of a SIDA was detected for oil and cereals. In addition, several food matrices have been analyzed for the compounds under study.

Keywords: *Phytosterol fatty acid esters, LC–MS/MS, validation*

D-30
CAROTENOIDS CLEAVAGE ACTIVITIES IN CRUDE ENZYME EXTRACT FROM PANDAN LEAVES (*PANDANUS AMARYLLIFOLIUS*)

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Carotenoid degradation products or called as norisoprenoids are known as aroma impact compounds in several plants 1. C13 norisoprenoid e.g. β-ionone with its fruity and violet like characteristics has been used in several application in food and beverage industries2. Nowadays, consumer preference is higher to the “organic” or “bio” products, where the “natural” β-ionone has a market value approximately 10–100 times higher than synthetic form. Formation of norisoprenoids from carotenoids have been investigated recently by enzymatic reaction from its native source e.g. in quince, starfruit, nectarine, tea, seaweed, etc. Pandan wangi is a common name of a shrub called Pandanus amaryllifolius. The genus name, Pandanus, is derived from the Indonesian name of the tree, pandan. The leaves from the plant is used for several general purposes e.g. flavours, natural colorants and traditional treatments. The general objective of this research are to determine the enzymatic carotenoid cleavage activities by using two different carotenoids substrate (β-carotene and β-apo-8’-carotenal), to determine the influence of pH and temperature to the enzymatic activities and to determine the enzymatic reaction product determination by using non solvent technique Head Space Solid Phase Microextraction Gas Chromatography (HS–SPME–GC–MS). We found carotenoid cleavage activity in crude enzyme from pandan leaves by using two different carotenoid substrates (β-carotene and β-apo-8’-carotenal). The optimum pH of crude enzymes from pandan leaves is approximately 6, while in the optimum pH for carotenoid cleavage activity in tea leaves is approximately 7.4 and in star fruit is approximately 8.5. The optimum temperature of crude enzymes is 70°C. CCDs from several native sources also have quite higher optimum temperature, e.g. in quince (50°C), nectarines and starfruit (45°C), in tea (70°C), in seaweed (55oC) 4,5,6,7,8. We identified β-ionone as the major volatile reaction product isolated from the incubations of two different carotenoids substrate (β-carotene and β-apo-8’-carotenal) with isolated crude enzymes from pandan leaves by HS–SPME GC–MS.

Keywords: *Carotenoids, C13 Norisoprenoids, Pandan Leaves*

Acknowledgement: *OeAD and Institue of Food Science, BOKU*

D-31
CAROTENOIDS BREAKDOWN PRODUCTS: THE NORISOPRENOIDS IN PANDAN LEAVES (*PANDANUS AMARYLLIFOLIUS*)

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Degradation of carotenoids yield carotenoid derived aroma compounds or called as norisoprenoids which can exhibit powerful aroma properties as natural flavors. The interest of consumer demand for natural flavors has market value higher rather than synthetic flavors2. Several norisoprenoids are known as aroma impact compounds, e.g. the fruity signature of β-ionone is recognizable even at concentrations as low as 0.007 ppm, the flower signature of β-damascenone is recognizable even at lower concentrations as low as 0.002 ppm. In Indonesia, several herbal leaves have been use for condiments to natural colorants and also to improve aroma and flavor in foods e.g. pandan leaves. Pandan leaves, commonly known as pandan, are often used: (1) to give a nice color, refreshing, fragrant flavour to both sweet and savoury South-East-Asian dishes; (2) to imitate the more expensive aromatic Basmati and Jasmine rices so that pandan leaves are generally used in cooking ordinary non-aromatic rice and (3) to treat traditionally several illness in Indonesia. In this research the general objective is to determine norisoprenoids and carotenoids as the precursors of norisoprenoids. Several norisoprenoids were identified in pandan leaves e.g.β-ionone, alpha-ionone, β-cyclocitral by using Head Space Solid Phase Microextraction Gas Chromatography (HS-SPME GC-MS). Several carotenoids which can serve as precursors to the norisoprenoids e.g. β-and alpha-carotene, were identified in the same pandan leaves by using Photo Diode Array High Performance Liquid Chromatography (PDA HPLC) based on their HPLC retention times and spectral characteristic. β-Cyclocitral can be formed from oxidative cleavage of the double bond between carbons 7 and 8 of alpha- and β-carotene In similar fashion, β-ionone can be formed from the same carotenoid by oxidative cleavage of the double bond between carbons 9 and 10 and between carbons 9' and 10' for β-carotene. Although there are several possible precursors for alpha-ionone, alpha-ionone can only be directly formed from the oxidative cleavage of the double bond between carbons 9' and 10' of alpha-carotene.

Keywords: *Carotenoids, Norisoprenoids, Aroma compounds, Pandan Leaves*

Acknowledgement: *OeAD and Institute of Food Science, BOKU*

D-32
INFLUENCE OF MICROWAVE-ASSISTED EXTRACTION ON THE PROFILE OF FLAVONOIDS FROM ORANGE CV. PERA

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Flavonoids are the largest group of phenolic compounds and their structure consist of 15 carbons with two aromatic rings connected by three carbons (C6–C3–C6.). Citrus are significant sources of flavonoids, mostly flavanones, being hesperidin (hesperitin-7-O-rutinoside) the major one. Microwave-assisted extraction (MAE) has been noted for achieving a rapid heating of the sample-solvent mixture, accelerating the mass transfer of the target compounds from the matrix to the solvent. Using a new MAE method developed in our laboratory for determination of phenolic compounds, the present work aims to evaluate by HPLC–DAD the composition of flavonoids from orange [*Citrus sinensis* (L.) *Osbeck cv. Pera*]. Furthermore, to assess possible changes induced by MAE, the flavonoid profile was compared to that obtained by conventional liquid extraction. After extraction in the microwave, the extract was centrifuged and the supernatant was collected in a volumetric flask, filt ered and injected into the HPLC. The phenolic compounds were separated by HPLC on a C18 Synergi Hydro column using a linear gradient of water and acetonitrile, both containing 0.5% of formic acid. The relative percentage of hesperidin was > 88 % in the extracts obtained by both MAE and conventional extraction. The hesperidin content obtained by MAE was 646.68 mg/g fresh weight (FW) and by the conventional extraction was 660.74 mg/g FW, with no significant differences at 95% confidence between both values. Although bellow the quantification limit, other minor compounds identified were apigenin-6,8-C-hexoside (1 %) and naringenin-7-O-neohesperidoside (5 %). The new method using MAE does not modify the profile of flavonoids from orange.

Keywords: *Flavonoid, microwave-assisted extraction, HPLC–DAD*

Acknowledgement: *CAPES and FAPESP (2013/07914-8)*

D-33
VITAMIN B COMPLEX DETECTION IN INFANT FORMULA BY LC/MS/MS

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Vitamin B complex is a group of water-soluble vitamins that play important roles in cell metabolism, absence of individual vitamins in a diet can lead to several conditions including depression and high blood pressure so they are often added to foods especially infant formula. Vitamin B is a complex mixture of different compounds each structural different. Traditionally individual methods have been used to screen for each vitamin B so one method that is capable to screen for several vitamin B compounds in a single analysis would be beneficial. Here we present some new data acquired by LC/MS/MS with a screening method which contains all the major forms of Vitamin B. The required detection limits vary greatly between each vitamin B and range from low parts per billion to low parts for million levels. The method has therefore been developed to detect all the vitamins in the required ranges and has meant that some transitions have had to be detuned to maintain their linear response and enable one simple extraction for all. The LC/MS/MS uses reverse phase chromatography and positive mode electrospray ionisation and meet the requirements of all the limits of detection. The mass spectrometry methods utilises Scheduled MRM™ and a small particle size HPLC column. NIST reference material was extracted and then simply diluted and analysed by LC/MS/MS to show the applicability of this method to routine sample analysis.

Keywords: *Vitamins, nutrition, LC-MS/MS, multi-analyte*

D-34
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR DETERMINATION OF SELECTED METHYLXANTHINES AND BIOGENIC AMINES IN FOOD AT VARIOUS STAGES OF PROCESSING

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Methylxanthines and biogenic amines are one of compounds for which determination of content in food could be relevant to product's shelf life and human health. Excessive intake of methylxanthines has negative consequences due to the stimulating effect of caffeine and theobromine. Determination of content of biogenic amines in food is important because of potential "adverse" biological effect caused by the presence of selected catecholamines (tyramine) or indolamines (serotonin) in the products, which in high concentrations may be toxic. A special role is played by studies of amines in food products eaten in various forms, since it is possible to use amines as biomarkers of food freshness. High performance liquid chromatography is widely used among other analytical techniques in food analysis. Mainly because of ability to simultaneous determination of a large number of compounds which belong to different groups. The processes involved in food processing operations consist of mechanical and thermal properties. The most important mechanical operations includes grinding, mixing and dispensing. Thermal processes used in food processing are primarily designed to consolidate and preserve their products. These include pasteurization, blanching, conching, cooking, baking, frying and freezing. The study included cocoa beans from different countries later processed to chocolate bars and drinking chocolate as well as exotic fruits, which were processed into variety of preserves (juices, jams, sorbets, candied fruits). In each sample caffeine, theobromine, theophylline, and their metabolites (xanthine and monometyloksantyny) as well as biogenic amines (dopamine, tanephrine, tyramine, norepinephrine and serotonin) were determined. Analytical procedures for separation and determination of analytes in food samples were developed. The study was carried out using high performance liquid chromatography in reverse phase. Spectrophotometric (methylxanthines) and fluorescence (biogenic amines) detection was used. The elution of analytes was carried out in a gradient system. It was found that temperature processes have a significant impact on content of methylxanthines and selected biogenic amines in processed food. Correlation between processes and changes of contents of all compounds in samples of raw materials, semi-finished and finished products were determined. Developed procedures may be used in quality control process as well as implementation of new technological solutions.

Keywords: *HPLC, food processing, methylxanthines, biogenic amines*

D-35
THE ANTIOXYDATIVE PROPERTIES OF STEVIA REBAUDIANA

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Extraction of the herb Stevia Rebaudiana were studied for their antioxidants properties, the extractions were prepared from dry leafs and fresh leafs in order to examine the ability to maintain the antioxidants properties after different ways of drying process and several methods of extraction. Cluster analysis showed that the Stevia Rebaudiana herb is sensitive to the method of extraction. Therefore each infusion of the different method of extraction was examined in several analytical methods using titrations, UV-VIS spectrophotometer absorbance and HPLC. Significant differences were observed among the herbal different infusions. Cold extractions showed higher antioxidants properties, then worm extractions. Infusions of fresh leaf maintained their antioxidants properties better then dry ones. We found great interest in the study of antioxidants properties in Stevia derivatives sweeteners, there for solutions containing all known stevia derivatives sweeteners were tested for their antioxidants properties in the same analytical methods. Surprisingly, some of the solutions containing only the stevia derivatives sweeteners, showed antioxidants properties as well.

Keywords: *Antioxydative, Stevia rebaudiana*

D-36
IDENTIFICATION AND QUANTIFICATION OF ISOFLAVONES AND ANOTHER PHYTOCHEMICALS IN THE SOY BASED NUTRACEUTICAL BY UHPLC-ORBITRAP-MS

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Nutraceuticals products are a recent class of dietary supplements whose consumption has increased in the last few years. One of the main problems related to the development of this type of products is the existence of fraudulent activities as well as there is no standard global regulatory system. Food and Drug Administration (FDA) approved the law Dietary Supplement Health and Education Act (DSHEA) in 1994, which indicates that these products do not need FDA approval prior marketing because the companies are responsible for its safety [1]. Moreover, under the FDA Final Rule 21 CFR 111, companies involved during the manufacturing, packaging, labeling or hold dietary supplements must comply with the Dietary Supplement Current Good Manufacturing Practices (cGMPS) for quality control [2]. One of the products most widely consumed as those derived from soybean (*Glycine max*) or soy based products, which are widely used because its nutritional and health-promoting benefits. Great attentions have received isoflavones, which are found at high concentrations in soy products, as genistein, daidzein and glycitein. In order to ensure the quality of this type of products, there is an increasing interest for the development of new methods for the characterization of soybean phytochemicals components. Due to the complexity of the nutritional product matrixes, the use of chromatographic techniques coupled to high resolution mass spectrometry analyzers is a suitable tool. In this study, a method based on liquid chromatography coupled to Orbitrap has been developed for simultaneous detection and identification of phytochemicals in nutraceuticals from soybean. The use of Orbitrap allows full scan accurate mass data acquisition under electrospray ionization mode up to a resolution of 100,000 full with at half maximum (FWHM). Moreover, all ion fragmentation (AIF) mode allows the acquisition of characteristics fragments, which can be used for the identification of phytochemicals. The extraction conditions were optimized and the final extraction method consisted on a solid-liquid extraction procedure with 0.150 g of sample, using 30 mL of a mixture of methanol:water (80:20 *v/v*), followed by 100-fold dilution of the extracts with mobile phase (ammonium acetate 30 mM, pH 5:methanol, 50:50, *v/v*) in order to avoid matrix effect. Solvent calibration was used during quantification process. The chromatographic running time was lower than 48 min in order to separate the isomer compounds. The method was validated in terms of recovery, precision, linearity and suitable validation parameters were obtained. The developed method was finally applied in order to evaluate the quality of this type of products.

[1] S.H. Zeisel, Science 285 (1999) 1853.
[2] http://www.fda.gov/Food/DietarySupplements/default.htm

Keywords: *Isoflavones, liquid chromatography, food quality, Orbitrap, mass spectrometry*

Acknowledgement: *The authors gratefully acknowledge the Spanish Ministry of Economy and Competitiveness (MINECO) and FEDER (project ref. CTQ2012-34304).*

D-37
BIOACTIVE PEPTIDES GENERATED BY IN VITRO GASTROINTESTINAL DIGESTION OF DRY-CURED HAM

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Cured meat products are an important part of total processed meat products, among which dry-cured hams are mostly appreciated by consumers. Nevertheless, the generally high salt content partially affects their nutritional value, as the excessive consumption of salty foods is related to the onset of cardiovascular disease and hypertension. Reduction of salt content of cured meat products is a potentially interesting goal, but it is difficult to achieve without impairing the safety and the quality of the product. On the other hand, although the excessive consumption of meat products is indicated as a risk factor for certain diseases, it has been also demonstrated that proteolysis positively influences the digestibility of the products and generates peptides with interesting biological activities (antioxidant, antihypertensive), which may potentially counteract the negative effects. In recent years, a considerable amount of research has focused on the release o f bioactive peptides encrypted in food proteins, eventually to be considered as functional food ingredients aimed at health maintenance. The aim of this work is to study the peptide profile obtained after simulated gastrointestinal digestion of dry-cured Parma hams, identifying the peptide sequences, and to evaluate the bioactivity of the released peptides (antioxidant and antihypertensive activity). In vitro simulated digestion has been performed mimicking all the composition and contact times of the different digestive steps and the obtained hydrolysates analyzed by LC–MS. Antioxidant activity as radical scavenging capacity and antihypertensive activity as ACE inhibiting activity have been measured. A comparison was made between hams characterized by different salt content, produced with new technologies aimed at lowering salt amount, and different ageing time. Statistical analysis showed that the digested samples can be grouped according to the ageing time (18–24 months) and that ageing time and salt content significantly affect the antioxidant and antihypertensive activity. Fractionation of the peptide mix ture has been performed in order to identify the most active peptides, which can be synthesized by solid phase synthesis in order to test their specific bioactivity and to investigate structure-activity relationships.

[1] E. Escudero, M.-C. Aristoy, H. Nishimura, K. Arihara, F. Toldrá, Antihypertensive effect and antioxidant activity of peptide fractions extracted from Spanish dry-cured ham. Meat Science 91 (2012) 306–311.
[2] Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F., & Staton, C. Bioactive peptides from muscle sources: Meat and fish. Nutrients, 3 (2011) 765–791.

Keywords: *Dry-cured ham, simulated gastrointestinal digestion, antioxidant activity, antihypertensive activity*

Acknowledgement: *AGER Project "Advanced research in genomics and processing technologies for the Italian heavy pig production chain"*

D-38
EFFECTS OF PROBIOTIC BIOMIN®IMBO ON PERFORMANCE AND ANTIOXIDATIVE STATUS OF BROILER CHICKS

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The effects of dietary supplementation of a probiotic BIOMIN®IMBO and coccidiostatic-salinomycine on performance and antioxidative status of broiler chicks were investigated. A total of seven hundred and fifty, one-day-old of both sexes (Cobb 500) were randomly assigned to three treatments with five replicates of fifty chicks based on a completely randomized design. The control group was fed basal diet and experimental groups was supplemented with probiotic BIOMIN®IMBO (*Enterococcus faecim* + fructo-oligosaccharides) in addition 1 g/kg and coccidiostatic salinomycine in addition 60 mg/kg to basal diet for 42 days, respectively. Diets supplementation with probiotic BIOMIN®IMBO and coccidiostatic- salinomycine increased body weight of broilers compared to control birds. Broilers fed probiotic statistically consumed more feed over the entire experimental periods. Feed efficiency improved slightly in different periods in supplemented groups compared to control birds. The serum glutathione peroxidase (GSH-Px), peroxidase (POD), glutathione (GSH), glutathione reductase (GR) and catalase (CAT) concentration were significantly higher, while there was a significant decrease (P

Keywords: *Broiler, probiotic BIOMIN®IMBO, performance, antioxidative status, coccidiostatic-salinomycin*

D-39
BIOLOGICALLY ACTIVE COMPOUNDS IN 71 ROSEHIP SAMPLES

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Rosehips are a rich source of many biologically active compounds. Besides the high content of ascorbic acid, rose hips have a significant content of other antioxidants, e.g., carotenoids (β-carotene and lycopene), tocopherols, flavonoids (catechin, rutin, quercetin…), triterpenoic acids (betulinic, oleanolic and ursolic acid), phytosterols, and many others. The high potential of rosehip's biologically active compounds can be used in food supplements.

In our study, a set of 71 rosehip samples (different varieties and taxa) supplied from Institute of Botany, ASCR were analyzed. The antioxidant activity (DPPH method) of samples ranged from 11 to 348 mg/g equivalent of ascorbic acid. The influence of storage condition (dried or frozen fruits) on antioxidant activity and vitamin C stability was monitored, the content of vitamin C (HPLC/UV) was higher in frozen samples (average 14 mg/g) compared to dried samples (average 9 mg/g). The major rosehip carotenoids (HPLC/DAD) were β-carotene (5 – 373 mg/kg) and lycopene (3 – 176 mg/kg). The average ratio of β-carotene to lycopene in analyzed samples was 1.6: 1. Rosehip samples were also analyzed for flavonoids and triterpenoic acids.

For metabolomic fingerprinting / profiling of 71 rosehip samples, a unique ionization source Direct Analysis in Real Time (DART) coupled with a High Resolution Time of Flight Mass Spectrometer (HR-TOFMS) was used. This technique enabled the identification of many compounds (both primary and secondary metabolites) occurring in rosehip extracts. The main identified compounds in rosehip peels are campherol, phloretin, eryodictiol, catechin, quercitin, taxafolin, myricetin, ß- sitosterol, triterpenoic acids, lycopene and ß- carotene. The content of biologically active compounds and also metabolomic profiles differs significantly in individually tested varieties and taxa.

Keywords: *Rosehips, Antioxidant Activity, Vitamin C, Carotenoids, Metabolomic Profiling*

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FLAVOURS AND ODOURS

(E-1 – E-19)

E-1
CHARACTERIZATION OF FLAVORED TOBACCO WITH GC×GC–TOFMS AND GC×GC–HR–TOFMS

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Flavored cigarettes (except menthol), considered "starter products" targeted towards youth, were banned under the Family Smoking Prevention and Tobacco Control Act in 2009. Other flavored tobacco products can still be purchased, but regulations for these are under consideration. Detecting individual flavor compounds within a complex tobacco matrix can be accomplished with chromatographic separations to isolate individual analytes and mass spectral detection for identification. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOFMS) offers rapid characterization of tobacco products and their flavor analytes. Analysis with high resolution TOFMS provides accurate mass information to verify analyte identification. The samples of flavored and unflavored tobacco samples were analyzed by headspace SPME and GC×GC–TOF MS technique. Full mass range TOFMS data were collected which allowed for measuring targeted flavor additives and also for detecting non-targeted naturally occurring flavor analytes present in the tobacco samples. Compared to one-dimensional GC–TOF MS, more compounds could be detected by GC×GC thanks to the employment of two different column selectivities within one run. ChromaTOF software was used for automated peak finding, spectral deconvolution, combination of slices and quantification. Main aroma components were reliably identified both in natural tobacco and flavored tobaccos. Due to similarities in mass spectra, identification based on NISTMS library comparison may not always be reliable. The analysis was therefore performed in parallel by GC×GC-high resolution TOF MS, providing the combination of efficient chromatographic separation with unrivalled resolution of 50,000 and mass accuracy below 1 ppm. This technique allowed additional highly reliable identification of unknown compoun ds.

Keywords: *GC×GC, TOF MS, flavored tobacco, high resolution TOF MS*

E-2
HOPS AROMA ANALYSIS RELEASED AT DIFFERENT BOIL TIME BY HS-SPME SAMPLING AND GC–TOF MS AND GC×GC–TOFMS

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Monitoring of flavors associated with food products is important in the beverage and food industry. Ensuring consistency in flavors can provide a measure of quality control and changes in the flavor and aroma notes can be determined that relate to processing methods or quality. This information can be used to control modifications in optimizing a food item or a production method. Flavor profiles are comprised of the volatile and semi-volatile compounds that contribute to the characteristic aroma of a food and generally consist of a large number of compounds that span a range of concentrations. Hops are one of the main ingredients used in beer-brewing and serve as both a natural preservative and as a flavoring agent. The leafy green flowers are responsible for the characteristic bitterness in beer, but also impart other perceptions such as floral, tangy, piney, or citrusy notes. The timing of the hop addition can have a large impact on the eventual aroma and flavor profile of the final product. In this poster, a method was developed to characterize aroma and flavor compounds associated with hops (*Humulus lupulus*), throughout the boiling stage of the beer brewing process. The boil process was simulated and headspace solid phase micro-extraction (HS–SPME) was used to sample the volatile and semi-volatile aroma and flavor compounds in the headspace of a boiled hop flower extract. Both one- and two-dimensional gas chromatography (GC and GC×GC) with Time-of-Flight Mass Spectrometry (TOF MS) were subsequently used to separate, quantify, and identify these compounds. Target analytes were monitored throughout the boil and quantified in order to determine aroma and flavor changes as a function of boil time.

Keywords: *Hop, flavor, aroma, GC×GC, TOF MS*

E-3
HONEY SWEET MEDICINAL DRUGS

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Apiarists let prepare by bees honey products containing medicinal drugs. Our aim was to prove that the active ingredients originated from the herbs are also present in the honey products. We analyzed the volatile components of honey consisting of medicinal plants. The characteristic compounds of plant leaves, berries, flowers were found in honey, therefore it was obvious that the components responsible for the medicinal effects (if they are not identical with the volatile components) were also transferred to honey. The components from the syrupy and honey samples obtained by SPME were analyzed by GC and GC–MS. The fingerprints were compared to those stored in a database and the similarity was reported. The syrupy samples were prepared from peppermint (Mentha x piperita) leaves, stinging nettle (*Urtica dioica*) leaves, wild rose (also known as dog rose) (*Rosa canina*) berries, black elderberry (*Sambucus nigra*) berries, scented mayweed or chamomile (*Matricaria recutita*) sprout, smallflower hairy willowherb (*Epilobium parviflorum*) leaves, common medlar (*Mespilus germanica*) fruits, and garden sage (*Salvia officinalis*) leaves. In our present study we analyze some groups of non-volatile components (color compounds, polyphenols) from the plant (if it was possible), from syrupy samples prepared for feeding the bees and from honey. Like in the case of volatile components the characteristic non-volatile components show high similarity compared the honey and the corresponding syrupy samples. Those honey products are commercially available and they are labeled as „TÖBBMINTMÉZ" (MORETHANHONEY).

[1] HARANGI, J., BALÁZS-HAJDÚ, I., BABKA, B., TAKÁCS, T., NAGY, É and PROKISCH, J., 2012. Mézédes gyógyhatású készítmények elemzése. Annual Meeting, 2012. Hajdúszoboszló: Hungarian Society for Separation Sciences.

Keywords: *Honey, medicinal effects, biological active components*

E-4
DETERMINATION OF VOLATILE COMPOUNDS IN HERBS AND TEAS OF THYME

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In the kitchen, thyme is used as an aromatic spice. A tea infusion of thyme is also applied for medicinal purposes in the treatment of colds and coughs. Both, the characteristic thyme aroma and the pharmacological effects can be attributed to the volatile compounds [1,2]. Consequently, they are an important criterion in the quality control of thyme and should be analyzed. The volatile compounds in eight thyme-herbs and two thyme-teas from the retail trade were determined and compared with regard to their composition. According to the GC–MS profiles obtained, the samples were divided into two groups, a thymol-rich group and a carvacrol-rich group. The concentrations of volatile compounds measured in the samples varied considerably. However, there was no relationship between the content of volatile compounds and the price of the analyzed products.

[1] Stahl-Biskup and Sáez, CRC Press 2012, ISBN 10.0-415-28488-0
[2] Basch et al., J. of Herbal Pharmacotherapy 2004, 4(1), 49–67

Keywords: *Volatiles, thyme, herbs, GC–MS*

E-5
AROMA ACTIVE COMPOUNDS OF ORANGE JUICE FROM THE EXTRACTION STEP OF FCOJ PROCESSING

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Brazil is the world leader in orange production and exportation of frozen concentrated orange juice (FCOJ). Sensory changes that occur during the FCOJ processing may lead to the loss of aroma and flavor characteristic of the natural juice and influence consumer's acceptance. The aim of this study was to determine the odoriferous importance of the compounds for the aroma of the fresh orange juice. The juice was from Pêra-Rio orange, obtained from the extraction step of FCOJ processing. The juice was supplied by a citrus industry from Araraquara, SP, Brazil. Three liters of orange juice from the extraction step were collected in the 2012 harvest. The juice showed soluble solids content of 9.6 oBrix. The volatile compounds were isolated using solid phase microextraction (PDMS/CAR/DVB, 50/30 µM coating), separated by high resolution gas chromatography and identified by gas chromatography-mass spectrometry. The compounds odoriferous contribution was evaluated using the OSME olfactometric analysis, using three trained and selected judges. Data concerning each aroma perceived during the olfactometric analysis were registered using a 10-point hybrid scale anchored with the terms "none", "moderate" and "strong" in the points 0, 5 and 10, respectively. Twenty odoriferous compounds were detected in the fresh orange juice, mainly terpenes described as "green papaya", "citric", "bug", "eucaliyptus", "green grass" and "mint", esters described as "sweet" and "rubber", and aldehydes described as "citric" and "eucaliyptus". The major odoriferous importance for the aroma of fresh orange juice were attributed to the terpene limonene, described as "sweet citric", besides decanal, described as "citric", and the ester ethyl butanoate, described as "guarana, sweet". The terpenes linalool, described as "eucaliyptus", citronelol, described as "bug", and three other non identified compounds, described as "eucaliyptus", "green papaya" and "bug", showed intermediate odoriferous importance. These volatile compounds were associated with the sweety and acid flavor of the fresh orange juice.

Keywords: *Olfactometry, aroma, orange juice, terpenes*

Acknowledgement: *This work has been financed by FAPESP and CNPQ*

E-6
EFFECTIVE ON-LINE SAMPLE CLEAN-UP AND ANALYTE ENRICHMENT TO ACHIEVE A QUICK AND EASY UHPLC–ANALYSIS OF HOP ISO-ALPHA-ACIDS IN BEER

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In the food and beverage industry, the necessity to isolate analytes from very complex matrices is a common business. Therefore sample preparation is a crucial aspect in chemical analysis of food and beverages. Different sample preparation techniques can be used to remove matrix or enrich analytes, with solid-phase extraction (SPE) being generally used when HPLC separation is applied. Typically SPE is performed manually, thus a significant bottleneck as well as error source in laboratory workflows. A model case is the brewing process of beers where very complex matrices form the basic samples which need to be analyzed in time due to the ongoing process in the brew kettle. However, a conventional analysis takes about an hour as a result of sample pretreatment steps, a manual SPE-procedure, and the RP-HPLC separation itself. An automation of this sample cleanup and analyte enrichment process eliminates all issues described above, while the speed-up capabilitie s of UHPLC technology can significantly reduce the time for the chromatographic separation. Hence, direct injection of untreated beer samples becomes feasible, ensuring higher confidence in the analytical result and higher throughput by unattended operation. In this presentation, the UHPLC separation of hop iso-alpha-acids in beer with an automated on-line SPE solution is illustrated. An untreated beer sample is injected directly; all SPE steps are performed automatically, and the whole analysis lasts only nine minutes. Thus, no kind of manual sample pre-treatment is needed anymore, and the result of the high-speed separation reflects the content of iso-alpha-acids in beer virtually in real-time. Using this automated on-line SPE RP-UHPLC approach, the bitterness, foam, and stability of a beer can be controlled in a quick and easy way. The prerequisites to achieve the necessary UHPLC performance are discussed in detail. This addresses the intrinsic challenges in the chromatog raphic match of stationary and mobile phase between SPE and LC, as well as the proper transfer of the analyte plug between the two steps.

Keywords: *UHPLC, hops, iso alfa acids, online SPE*

E-7
A NEW METHOD FOR THE EXTRACTION OF VOLATILE LIPID OXIDATION PRODUCTS FROM SHRIMP BY HS–SPME–GC–MS

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The short chain volatile products formed from the β-cleavage of hydroperoxides are generated during lipid oxidation and have great impact on the sensory quality of foods. In addition, analysis of these secondary lipid oxidation products presented good correlation with the more traditional analyses such as the peroxide index, TBARS and the sensory analysis. A new method based on headspace solid phase micro-extraction (HS-SPME) and GC-MS was developed, aimed at evaluating the formation of volatile lipid oxidation products (VLOPs) in shrimp during the salting and drying process. Fresh, whole (with head and shell) pacific white shrimp (Litopenaeus vannamei), were submitted to salting in boiling water (30% NaCl for 10 min) and drying in an air circulating oven at 60°C for 4 h. In order to establish the method used to extract the volatile compounds by SPME, three factors that affect the extraction performance were evaluated in sequence: the type of stationary p hase used to coat the fiber; the sample preparation method and the extraction time/temperature binomial, using the best condition obtained in the previous step for the subsequent step. The fiber coated with DVB.CAR.PDMS was the most adequate for the quantification of the VLOPs. The best analytical conditions were obtained by homogenization in a turrax followed by extraction at 40°C for 30 minutes. The optimized method allowed for the rapid and simple extraction of the VLOPs, with low detection (≤ 0.15 $ng.g^{-1}$) and quantification (≤ 0.50 $ng.g^{-1}$), limits, and high precision (≤ 12.67%) and extraction efficiency (≥ 96.11%). 69 different compounds could be identified in salted, dried shrimp, and 55 were tentatively identified. Among the compounds identified, 14 could be classified as VLOPs: pentanal; hexanal; 1-penten-3-ol; heptanal; 2-pentylfuran; 1-pentanol; octanal; 1-hexanol; nonanal; 1-octen-3-ol; 1-heptanol; decanal; 1-octanol; 2-octen-1-ol. In conclusion the proces s negatively affected shrimp quality, increasing the volatile oxidation products, especially hexanal.

Keywords: *Lipid oxidation, Litopenaeus vannamei, volatile compounds, pacific white shrimp, validation*

Acknowledgement: *CNPq, Fapesp*

E-8
EFFECT OF THE SEQUENCE OF THERMO-MECHANICAL TREATMENTS IN THE AROMA PROFILE OF VEGETABLES

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Thermal treatments are widely used in food industry. They produce a softening of vegetable tissues by β-elimination of pectin and consequent debilitation of the intercellular connexions. Application of a shear treatment before or after the thermal process will determine the pattern of tissue disruption and, therefore, result on different structures. The main objective of this study is to determine the effect of these thermo-mechanical processes on the volatile profile of vegetables. Different temperatures and time regimes and comparative flavour analysis of these samples were performed. Understanding the change of flavour profile and microstructure as a function of different process conditions is crucial to develop better products.

Keywords: *Flavour analysis, vegetable purée, thermo-mechanichal treatment, volatiles*

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E-9
A COMPARISON OF SORPTIVE EXTRACTION PHASES FOR THE ANALYSIS OF TAINTS AND OFF-FLAVOURS

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A taint in food results from contamination by a foreign chemical from an external source whereas off flavours result from internal deterioration of the food. The presence of compounds causing taints or off-flavours in food is a major concern to the food industry. Following the occurrence of a food taint, it is imperative that robust analytical procedures are followed to enable identification of the compound(s) responsible and identify root cause. The compound(s) responsible for the taint may be present at extremely low levels (sub ppb) and in some cases specialist extraction techniques may be required to enable detection. The use of stir bar sorptive extraction (SBSE) with PDMS coated Twisters™ for determination of food taints has been reported previously [1]. The use of the new ethylene glycol modified silicone (EG-Silicone) sorbent phase Gerstel Twister™ and silica based active carbon MonoTrap™ (Hichrom Ltd) were evaluated for extraction of a number of compounds known to cause taints. Example compounds were chosen based on previously reported taints from a range of origins and included those most commonly investigated (such as halogenated phenols and anisoles). Initially the efficiency of extraction was evaluated by direct immersion into model systems (spiked water) followed by thermal desorption GC-MS. Subsequently techniques were tested on some real life examples of taint issues from the food industry. Both Twisters™ were observed to extract all the analytes of interest. Results indicate an increase in extraction efficiency for some compounds using the new EG-Silicone sorbent phase compared to PDMS. The silica based active carbon MonoTrap™ also extracted the majority of compounds evaluated, but did not offer additional enrichment or improve extraction efficiencies compared to the PDMS coated Twisters™.

[1] Ridgway, K., Lalljie, S. P. D. and Smith, R. M., Analytica Chimica Acta 677(1)(2010) 29-36

Keywords: *Taints, Twister, sorptive extraction*

E-10
WILD YEAST DURING SPONTANEOUS FERMENTATION OF WINE

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Low-intervention winemaking methods based on spontaneous fermentation are becoming more popular among wine producers and consumers. Some wine producers and viticulturists have re-adopted traditional winemaking methods to generate unique attributes that differentiate their products, improve wine quality and increase the variety of complex flavors that characterize regional vineyards. In spontaneous fermentation of wine, the grape must is fermented by the wild flora. Wines with unique and distinctive characters can be obtained. However, longer fermentation times, higher risk of stuck or sluggish fermentations and of off-flavor formation can occur, and thus, the risk of economic losses is high. The aim of this work is to establish the basis for the development of a cost effective detection kit for on-site monitoring of wild yeasts in spontaneous fermentations. In addition, suitable chemical markers for objective identification of the wine origin will be determined. It is expected that this device allows the opportune detection of problematic fermentations and the incorporation of adequate solutions to avoid off-flavor compounds and defective wines. We studied the dynamic behavior of wild yeasts during spontaneous wine fermentation at a winery in the Valais region of Switzerland. Wild yeasts in the winery environment (i.e. on the grape surface, in the vineyards and in the cellar) were characterized using a novel polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method. Up to 11 different yeast species could be isolated from the vineyard air, whereas only seven were recovered from the surface of the grapes. We found that the yeasts Metschnokowia pulcherima, Rhodotorula mucilaginosa, Pichia kluyveri, P. membranifaciens and Saccharomyces cerevisiae remained active at the end of the fermentation process. We also studied the dynamic behavior of yeasts in Qvevris (amphora-like clay vessels) for the first time using a novel, highly-sensitive quantitative real-time PCR method. We found that non-Saccharomyces yeasts were present during the entire fermentation process, with R. mucilaginosa and P. anomal a the most prominent species. We studied the relationship between the predominance of different species and the output of the fermentation process. We identified so-called spoilage yeasts in all the fermentations, but high levels of acetic acid accumulated only in those fermentations with an extended lag phase. The fermentation parameters and production of volatile compounds varied from specie to specie and from one mixture to the other. Polyclonal antibodies from the predominant species found to affect the wine are being developed to be included in the kit prototype. The antibodies specificity and cross-reactivity is under evaluation. Statistical analysis and mathematical models will be applied to the volatile compounds HS–GC–MS data to identify distinctive compounds enabling to discriminate or classify the different wine musts.

Keywords: *Wine, spoilage, amphora*

E-11
ENHANCED ODOUR PROFILING BY TD/GC×GC–TOF MS WITH SELECTIVE IONISATION

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Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF MS) offers greatly enhanced peak capacity, through the coupling of two columns of different selectivity, as well as highly sensitive detection and definitive mass spectral identification of trace-level analytes. The combination of this technique with thermal desorption (TD) for the collection and pre-concentration of volatile and semi-volatile organic compounds makes it the ideal choice for the analysis of complex flavour and fragrance samples. Despite the superior separation afforded by GC×GC–TOF MS, the identification of individual compounds in complex samples may be further complicated when similar mass spectral characteristics are evident across entire chemical classes, such as with the monoterpenes. Recent advances in ion source technology aim to solve this problem through the ability to switch between hard and soft electron ionisation with no inherent loss in sensitivity. This poster will introduce Select-eV, a ground-breaking development in electron ionisation, which offers a wide, tuneable range of ionisation energies with no requirement for source switching or additional reagent gases. In fact, Select-eV soft ionisation has actually been shown to increase the sensitivity and selectivity of GC×GC–TOFMS. The ability to provide enhanced molecular ions whilst retaining structurally-significant fragment ions allows for greater orthogonality between the mass spectra of isomeric compounds, thus simplifying compound identification. TD can bring the added advantage of retaining part of the sample for re-analysis at complementary ionisation levels; allowing confident and secure repeat analysis, especially when combined with tagged TD tubes which have the ability to store valuable information on the provenance of each sample. This poster will illustrate the increased dimensionality of TD/GC×GC–TOF MS with selective ionisation, for robust characterisation of complex mixtures as required by the flavour and fragrance industry.

Keywords: *Thermal desorption, GC×GC–TOFMS, odour profiling, soft electron ionisation*

E-12
AROMA-ACTIVE COMPOUND IDENTIFICATION AND SENSORY CHARACTERISTICS OF THAI MALE GIANT WATER BUG, *LETHOCERUS INDICUS* (*LEP.* AND *SERV.*)

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Giant water bug (*Lethocerus indicus*) or 'Maengdana' in Thai, is used in some types of traditional Thai cuisine and neighbor countries for adding a unique aroma to food products. There are two types of male giant water bugs available in the local markets; frozen fresh bugs (FFB) and salted boiled bugs (SBB). Nowadays, availability of the giant water bug is scarce in nature due to a decline in available habitat and other environmental factors, while the need for its use as a flavor is constantly increasing. Moreover, studies concerning their key aroma compounds contributing to the overall aroma are also limited. Identification of the key aroma compounds of natural male bugs is necessary for creation of a flavor essence that closely mimics that of the natural scent for the uses in cooking. Therefore, direct solvent extraction/solvent-assisted flavor evaporation (SAFE) followed by gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GCO) and aroma extract dilution analysis (AEDA) were performed to identify the aroma-active compounds of FFB and SBB male giant water bug samples. Esters, especially (E)-2-hexenyl acetate and (E)-2-hexenyl butanoate, which contributed banana-like odors, were the predominant odorants and also showed the highest concentrations in both extracts of the bug samples. For ranking of aroma potency, odor activity values (OAVs) were calculated for both bug samples. On the basis of AEDA and OAVs results, the most potent odorants of the bugs were lipid-derived compounds, especially of the abundant ester - (E)-2-hexenyl acetate, butanoic acid with fecal-like odor, and (E)-2-hexenoic acid with a sweaty odor. In addition, 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline with popcorn-like odor were identified only in SBB sample. Aroma comparison of reconstitution model was performed and compared with the original aroma of the bug sample. Sensory descriptive analysis indicated that floral and green apple odors were the dominant odors in both of the model and original bug sample. According to discriminative test with the R-index ranking test, omission of (E)-2-hexenyl acetate from the model gave change in overall aroma of the model. Furthermore, banana-like attribute was found to be significantly different ($p < 0.05$) between the complete model and the models from which either single or group of esters was omitted. This indicated that (E)-2-hexenyl acetate was the powerful character-impact odorant of this bug and it was conformed to the finding from the instrumental analyses.

Keywords: *Giant water bug (Lethocerus indicus), GCO, AEDA, aroma reconstitution model, omission stud*

Acknowledgement: *The authors would like to acknowledge the Royal Golden Jubilee Scholarship (Grant No. PHD/0077/2549) from Thailand Research Fund for funding support, Suranaree University of Technology, Thailand and University of Illinois at Urbana-Champaign, IL, USA for technical support.*

E-13
MAY BRYNDZA CHEESE: STUDY OF VOLATILE AROMA-ACTIVE COMPOUNDS FORMATION

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Bryndza cheese is a traditional food product of the mountain regions of Slovakia. It maintains its position in the market in Slovakia and, to a certain extent, also in neighbouring countries (Czech Republic, Hungary, Austria). May bryndza cheese is the highest valued variant of bryndza, due to its distinctive flavour. It is produced in the beginning of summer season, in May. The time of production is believed to positively influence the quality of May bryndza cheese, probably by the quality of ewes' milk as influenced by the spring pastures. May bryndza cheese is produced from ewes' curd, which had been produced from raw ewes' milk and underwent short ripening for 10–14 days at 17–23°C. Ewes' curds are processed by milling with salt solution, in order to obtain the specific creamy texture of bryndza. The final product is stored under refrigeration up to 6°C and should be consumed within two weeks. The flavour and aroma of May bryndza cheese are apparently composed from compounds contained in ewes'milk and the products of fermentation by bacteria and fungi. The main fermentation product is lactic acid. The contribution of microflora to the production of typical aroma compounds may be limited due to the short time of ripening. Because of the need to characterize the organoleptic quality of May bryndza cheese as a high-value traditional food product, aroma-active volatile compounds were analysed in this study. Solid phase microextraction was utilized to isolation of volatile fractions, and coupled to gas chromatography – olfactometry (GC/FID–O), and gas chromatography – mass spectrometry (GC/MS). Twenty-seven key volatile aroma-active compounds were found and described in the intermediate product, ewes' curd ripened for 0, 1, 2, 4 and 8 days, or in the final product, May bryndza cheese. A major overall increase in the number of aroma-active volatile compounds and in their odour intensity or concentration took place during the first few days of the ripening of ewes' curd, and the odour gradually culminated at the end of ripening of ewes' curd. During the final technological step of bryndza cheese production, when the ewes' curd ripened for 10 days was milled with NaCl solution, 8 aroma-active volatile compounds disappeared. The key aroma-active compounds of May bryndza cheese were acetic acid, ethyl acetate, 2-methylpropanol, 3-methylbutanal, 3-hydroxy-2-butanone, 3-methylbutanol, 2-methylbutanol, butanoic acid, 2,3-heptanedione, pentanoic acid, 2,4-dimethyl-1-heptene, ethyl hexanoate, tentative 4-methyl decane, 3-methylbutyl butanoate, 2-phenylethanol, tentative 4-octanone, octanoic acid, 2-phenylethyl acetate and one unknown compound.

Keywords: *Cheese - Ewe - Aroma - Gas chromatography – Olfactometry*

Acknowledgement: *This work was supported by the project of the Slovak Research and Development Agency APVV 0590-10.*

E-14
SPME–GC/MS AND ELECTRONIC NOSE ANALYSIS OF BALSAMIC VINEGAR

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Balsamic vinegar, a very widely used dressing, is made using grape must, vinegar and caramel with a seasoning of at least two months. Its chemical and organoleptic properties are determined by the raw materials used, the seasoning in wooden vessels and the technological procedures employed. 80 Protected Geographical Indication (PGI) balsamic vinegar were analyzed with two different techniques that do not require sample preparation, SPME-GC/MS and electronic nose. SPME-GC/MS analysis: carboxen-polydimethylsiloxane fiber, HP 6890 gas chromatograph with 5973 MSD detector, SPME autosampler, Supelcowax 30 m × 0.25 mm x 0.20 µm column; total ion mode from 30 to 550 amu, acquisition, 1 scan/s Electronic nose analysis: NST instrument, 4 ml of vinegar into a 20 ml glass vial capped with PTFE/silicone septum, 40°C for 30 min. Using SPME technique more than 60 compounds were detected: alcohols (isoamyl alcohol, phenyl-ethyl alcohol); aldehydes (furfural, 5-methyl-furfural, benzaldehyde, 3-methyl-benzaldehyde, acetoxymethyl furaldehyde); esters (ethyl acetate, methyl acetate, isoamyl acetate, phenylethyl acetate); acids in addition to acetic acid (esanoic acid, octanoic acid and dodecanoic acid). With electronic nose data unsupervised cluster analysis techniques (PCA, dendrograms) and classification supervised techniques (PLS–DA, LDA, SVM) were used. Attempts were made to distinguish among industrial and traditional made PGI balsamic vinegar and to recognize vinegars with added caramel.

Keywords: *Balsamic vinegar, SPME, electronic nose, chemometrics*

E-15
VOLATILE AROMAS AND MICROTEXTURAL ANALYSIS OF COFFEE BEANS DURING ROASTING PROCESS

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The chemistry of coffee flavor and aroma is highly complex and is still not completely understood. The chemical composition of coffee beans differ upon a wide range of factors, such as species and variety of bean, geographic origin, soil conditions, storage of the beans, roasting time and temperature. Roasting conditions (time and temperature) have a major impact on the physical and chemical properties of roasted coffee beans, and are precisely the variables that determine the final taste and aroma of coffee beans. For this reason, solid phase microextraction in the headspace coupled to gas chromatography-mass spectrometry (HS–SPME–GC/MS) was carried out in this study in order to analyze the impact of roasting time (10–30 min) and temperature (180–240°C). Roasted coffee beans at different conditions were milled and analyzed by HS-SPME. After an optimization of the extraction conditions (incubation time, temperature and sample amount) by means of experiment al design, samples of all over the world were quantified by multiple headspace extraction (MHE). MHE is a very useful and valuable approach when the total amount of volatiles is to be determined in a solid sample as it has been demonstrated in this work. When a coffee bean is exposed to higher temperatures than 200°C, many changes are taking place inside the bean which supposes changes in the microstructure and texture, and also extensive chemical reactions are induced. Due to the high pressure generated when the gases are released in the roasting process, structural changes occur. Physical changes as weight loss and color change have been measured and also structural changes have been studied. For this purpose Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray spectroscopy (EDX) have been employed. Coffee beans were divided in two and images of the inside were acquired. Image analysis has been employed for the quantification of the pores created while roasting and multivariate data analysis (PARAFAC and Tucker3) has been used in order to explain the elemental distribution of the beans after the exposure to heat. The structural changes together with the color change, the weight loss and the volatile compounds development previously quantified by HS-SPME were cautiously compared obtaining important significant relevance of the roasting process.

Keywords: *Coffee, volatile compounds, microtextural analysis, headspace*

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E-16
THE CHARACTERISATION OF PLANT-DERIVED FLAVOUR EXTRACTS USING LC–HRTOFMS AND SPME–GC–MS

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Plant-derived flavour extracts are inherently complex and present a significant challenge for analytical chemists to achieve separation and detection of components of interest. The extracts contain compounds with a large variation in abundance and chemical and physical properties. Part of our research is focused on understanding the constituents of plant-derived extracts. Test extracts were produced by the manufacturer after ethanol and water addition to the plant material followed by high temperature extraction or a cold maceration process. Flavour extracts analysed include those of the Chuan Xiong (Chinese lovage root), Illicium verum (Chinese star anise) and cinnamon species (Cinnamomum burmannii, Cinnamomum cassia, Cinnamomum loureiroi). In this study non-targeted profiling of plant extracts was completed using a Bruker maXis impact™ Q-TOF instrument (liquid chromatography high-resolution time-of-fight-mass spectrometer (LC–HRTOFMS) with diode array (DAD) detection and electrospray, atmospheric pressure chemical and atmospheric pressure photo ionization. The bulk chemical constituents that were identified were mono-, di- and polysaccharides, organic acids, alcohols, phenols and aldehydes. LC–HRTOFMS data were processed using the Bruker Dissect algorithm which detects all of the LC–MS components based on the chromatographic profile of the detected ions. Overlapping LC peaks were de-convoluted to assist with the identification of trace compounds and further characterise the plant-derived flavour extracts. Preliminary LC–HRTOFMS studies indicate the presence of over one hundred compounds in the extracts. The molecular formulae identified by the LC-HRTOFMS were confirmed by MS/MS or MSn experiments and further confirmation of compound identification was provided by the construction of an accurate mass library of flavour compounds. Additional characterisation of the flavour extracts was performed by applying solid phase micro-extraction–gas chromatography mass spectrometry (SPME–GC–MS). This enabled the identification of some of the more volatile compounds present in the extracts. Mass spectra of identified compounds were searched against commercially available mass spectral libraries to provide complementary information to the LC–HRTOFMS data.

Keywords: *LC–HRTOFMS, SPME–GC–MS, non-targeted profiling, flavour extract*

E-17
A NEW STABLE ISOTOPE DILUTION APPROACH FOR THE SENSITIVE QUANTITATION OF 3-METHYL-2-BUTENE-1-THIOL (MBT) IN BEER

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Beer, when exposed to sunlight, quickly develops an unpleasant aroma, commonly referred to as the "sunstruck-flavour". This off-flavour is predominantly caused by skunky smelling 3-methyl-2-butene-1-thiol (MBT), formed in the course of a light induced reaction involving isohumulone, riboflavin, and cysteine. Due to its extremely low odour threshold, even minimum amounts of MBT may have a detrimental effect on the aroma of beer. Therefore, a new method for its sensitive and reliable quantitation was developed. Using 4-vinylpyridine, MBT was converted to its pyridinylethyl derivative, which was finally analysed by LC-MS/MS. Following the stable isotope dilution approach, deuterium labeled MBT was used as the internal standard. The method was then applied to quantitate MBT in different beers before and after illumination.

Keywords: *MBT, beer, SIDA, sunstruck-flavour, 4-vinylpyridine*

E-18
DETERMINATION OF ANDROSTENONE AND SKATOLE IN PIG FAT BY HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED TO COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY–QUADRUPOLE MASS SPECTROMETRY

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The unpleasant odor released during cooking known as boar taint can be present in pig meat from uncastrated animals. Androstenone, a hormone produced by sexually mature animals is one of the responsible for this odor, as well as skatole, which is produced in the intestine. Both compounds accumulated in the fat tissues and lately disseminate into the bloodstream. In amounts higher than 1.00 and 0.25 µg/g, respectively, these compounds are already perceived by most consumers. Several analytical methods for their determination in pig fat have been proposed but most require complex sample preparation steps prior analysis. Solid Phase Microextraction (SPME) can be an interesting alternative for sample preparation, because the extraction and purification occur in a single stage. Also Comprehensive Two-dimensional Gas Chromatography (GC×GC) can be combined to SPME specially for complex samples due to its greater capacity of separating the analytes and increase in detectability. A saponification was used to decrease the matrix effect, and a Doehlert Experimental design was developed for the determination of the best extraction conditions. The validation was performed and the method was suitable for the quantitation of small quantities of skatole (less than 0.25 µg/g) with high precision and acccuracy, and allowed detection of androstone. The proposed method was employed succesfully in the determination skatole level and detection of androstenone in seven samples of pig fat.

Keywords: *Androstenone, GC×GC, Skatole, SPME*

E-19
FINGERPRINT ANALYSIS OF FLAVOUR COMPOUNDS IN A MOUNTAIN CHEESE BY GC–MSD AND HIGH RESOLUTION GC–TOFMS

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Kars kashkaval is a semi-hard Turkish traditional cheese with distinctive flavour characteristics. The manufacturing process of Kars kashkaval is unchanged during decades and it represents a core product in the agricultural economy of Kars province. The authenticity of this cheese variety is a matter of concern for the region. Therefore, the precise characterization of flavour profile of this cheese is important for the producers who want to claim the right for naming cheese manufactured in a specified restricted area. This presentation is the first reporting volatile profile of Kars kashkaval at different stages of ripening (1, 30, 60, 90 days). The samples of Kars kashkaval were taken from local dairy farms in Kars (Eastern Turkey). Also, regular matured kashkaval cheese samples were purchased from local market for the comparison of flavour profile. Headspace solid phase matrix extraction (SPME) combined with low resolution and high resolution GC/MS were applied to determine the volatile compounds. A total of 37 compounds that belong to a variety of chemical classes including aldehydes, ketones, esters, fatty acids alcohols, terpenes and hydrocarbons were detected and identified. The key-aroma compounds assumed as specific markers of manufacturing process have been located in the chromatograms and used to discriminate samples from other technological treatments. In the presentation, the study considering the identification of the chemical compounds characteristic for the particular type o f cheese will be undertaken. Advantages and limitations of both GC–MSD and high resolution GC–TOFMS systems will be discussed.

Keywords: *Flavour compounds, kashkaval cheese, SPME GC/MS analysis, HR–GC/MS*

Acknowledgement: *The authers would like to gratefully acknowledge Prof. Dr. Mitat Sahin from Kars Kafkas University for his help providing cheese samples.*

FOOD CONTAMINANTS (ENVIRONMENTAL)

(F-1 – F-54)

F-1
CHROMIUM (III) AND CHROMIUM (VI) CONTENT IN AGRICULTURAL PRODUCTS IN VICINITY OF CHROMIUM SMELTERS

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Chromium (Cr) is one of the most and important trace metals which can be present in two oxidation states: toxic Cr(VI) and non- toxic Cr(III). Cr(III) is an important microelement for plant and animal nutrition and essential for the maintenance of glucose as well as for the lipid and protein metabolism. With regard to human health, Cr(III) is a required nutrient, with 50–200 μg per day recommended for adults. On the contrary, Cr(VI) is toxic and carcinogenic for the human body, leading to lung cancer, skin allergy and probably also to asthma and renal diseases. A toxic effect for the biological systems is attributed to the ability of Cr(VI) to migrate across the cell membrane, thus enhancing the intracellular chromium concentration. Hexavalent chromium is rarely found in nature and is generally man-made, especially in fumes generated during the ferrochrome production. The permissible exposure limit (PEL) of chromium in air is 5 μg m−3 measured as Cr(VI). For this study, the samples of industrial dust, as well as samples of such agricultural products as corn, tomato and potato was collected. Samples of food were washed with deionized water for the Cr(VI) determination. All measurements were carried out using an Perkin Elmer atomic absorption spectrometer model AAnalyst 600 with Zeemann background correction. It was found that Cr(VI) concentration in industrial dust was rather high, in the range 120-320 μg g−1, which contaminates the surface of tomato. The internal concentration of Cr(VI) in tomato, corn and potato was about 0.6 ng g^{-1}. The reason for that could be the presence of antioxidant components in agricultural plants.

Keywords: *Cr(III), Cr(VI), ETAAS, Agricultural products, Chromium smelter*

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F-2
ANALYSIS OF POLYCHLORINATED BIPHENYLS (PCBs) IN FISH OIL SUPPLEMENTS BY GAS CHROMATOGRAPHY WITH HIGH RESOLUTION-TIME-OF-FLIGHT MASS SPECTROMETRY (GC–HR–TOF MS)

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Omega-3 fatty acids have many health benefits, such as decreased risk of cardiac dysrhythmia, decreased triglyceride levels and slowed growth rate of atherosclerotic plaque. These fatty acids are present in higher concentrations in fish, especially with high fat content, such as tuna and salmon. Therefore health associations recommend eating two servings of fish per week. Unfortunately, these same high fat content marine species are contaminated with persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) which are known to bioaccumulate in the food chain. Because of such environmental contaminants, the FDA recommends limiting the consumption of predatory fish that reside at the top of the food chain. Fish oil supplements from various suppliers were analyzed by gas chromatography and High Resolution Time-of- Flight Mass Spectrometry (GC–HRTOFMS) using novel multi-reflecting TOF technology. This data highlights the advantages of GC–HRTOFMS for the analysis of PCBs in extremely complex biological matrices. The ability of HRTOFMS to selectively extract masses at high resolution virtually eliminated background interferences attributed to the matrix which allowed both successful screening and trace level detection to be achieved.

Keywords: *Gas chromatography, high resolution TOF MS, PCBs, fish oil supplements*

F-3
GC×GC–TOFMS UTILIZED AS A BROAD-SPECTRUM ANALYSIS FOR ENDOCRINE DISRUPTOR COMPOUNDS IN URBAN AND RURAL WATERSHEDS

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A broad variety of chemical classes, encompassing drugs, pesticides, polymer additives, coatings materials, personal consumer products, industrial by-products and pollutants, belongs to the Endocrine-disrupting compounds (EDCs). There is worldwide concern over long-term environmental exposure to EDCs leading to serious health effects including a range of reproductive problems such as reduced fertility, male and female reproductive abnormalities, skewed male/female sex ratios, brain and behavior problems, impaired immune functions, and various cancers. This research presents a robust, broad range analysis for the detection of EDCs in impacted natural waters using Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (GC×GC–TOF MS). GC×GC facilitates enhanced detection, chromatographic resolution, and peak capacity while TOF MS allows very fast acquisition (up to 500 spectra per second) necessary to successfully acquire the data density needed to fully characterize extremely narrow GC×GC peaks. Thanks to improved sensitivity the combination of GC×GC and TOF enabled determination of low levels of targeted and untargeted compounds in complex samples. GC×GC–TOFMS analysis was conducted on multiple water samples from a rural and urban Midwestern U.S. watershed using a solid phase extraction. This research study presents a practical, robust, sensitive, and reliable method for the detection of EDCs in urban and rural watersheds. Methods for solid-phase extraction and GC×GC–TOFMS analysis were developed.

Keywords: *GC×GC, TOF MS, environmental, water, endocrine disruptor*

F-4
A METHOD FOR THE QUANTITATIVE DETERMINATION OF CADMIUM, LEAD, ARSENIC AND TIN IN MUSCLE BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP–MS)

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Metals such as Pb, Cd, and As are naturally occurring compounds, however their presence in the environment can also arise from both agricultural and industrial activities with animals often being exposed to these compounds through the ingestion of contaminated plants and soil. Exposure to these chemical compounds can lead to harmful effects and as meat and meat products constitute an important part of the human diet EU legislation requires that member states adopt appropriate surveillance measures regarding the presence of contaminants in live animals and animal products in accordance with Council Directive 96/23/EC. Commission Regulation (EC) No. 1881/2006 sets maximum levels for some of these contaminants in various foodstuffs. A method was developed and validated for the determination of cadmium, lead, arsenic and tin in meat, in accordance with Commission Regulation 333/2007, by Inductively Coupled Plasma Mass Spectrometer (ICP–MS). Meat samples were dig ested in a microwave oven using nitric acid, diluted with deionised water and analysed using an ELAN DRC-e ICP–MS, using Rhodium as an internal standard. Parameters such as limit of detection (LoD), limit of quantification (LoQ), specificity, recovery, repeatability, within-laboratory reproducibility, linearity, stability and uncertainty of measurement were evaluated. Detection limits for Cd, Pb and Sn were less than the required limits of 5 µg/kg, 10 µg/kg and 5mg/kg, respectively. Quantification limits were below the required limits of 10 µg/kg for Cd and Sn and 20µg/kg for Pb. For As the detection and quantification limits were less than 5µg/kg and 10 µg/kg, respectively. The method was shown to be very specific with no matrix or spectral interferences. Repeatability and within-laboratory reproducibility were assessed across four levels using the HORRAT criteria. Results for HORRATr and HORRATR values were less than 2. In addition to this the observed RSDr (repeatabilit y) and the observed RSDR (within-lab reproducibility) were shown to be less than 2/3 rds of the predicted RSD value across all levels for all elements. Linearity was established using 7 points over the range 5–500 ng/g for As, Cd and Sn, and 10–1000 ng/g for Pb. Correlation coefficients of $R^2 \geq 0.99$ were established for all elements. Commission Regulation (EC) No 333/2007 outlines criteria for the maximum standard measurement uncertainty allowed at all concentrations. For all the elements the calculated combined uncertainty was less than the maximum standard measurement uncertainty at the level of interest. Furthermore the accuracy of the method was assessed by participation in the 18th proficiency test on frozen meat organised by the EU-RL CEFAO Rome which resulted in Z-scores of 0.1 and 0.02 for Cd and Pb, respectively. In conclusion this is a precise and accurate method capable of high sample throughput, which has been fully validated and has met all method perform ance requirements of the European Commission.

Keywords: *Metals, ICP–MS, validation*

F-5
METHOD VALIDATION AND OCCURRENCE OF PCDD/FS IN FISH FROM BRAZIL

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An isotope dilution method for determination of 17 toxic dioxins (PCDD/F) in fish was validated for monitoring purposes. Samples were previously freeze-dried and then extracted using two different procedures: pressurized liquid extraction (PLE) with ASE 350® (ThermoFisher/Dionex) or Soxhlet extraction. In both systems hexane was used as the extraction solvent. Clean up was performed using acid silica gel column, followed by florisil column. Dichloromethane and hexane, respectively, were used as the elution solvents. After evaporation of the extract, recovery standards were added. Extracts were analyzed by GC-HRMS (AutoSpec® – Waters). Limits of quantification (LOQ) ranged from 0.05 pg/g (TCDD/F) to 0.40 pg/g (OCDD/F). The method was found to be linear in the range of 0.05 to 1.000 pg/g (TCDD/F). Trueness was assessed by analysis of CRM. The evaluation of precision (repeatability and within-laboratory reproducibility) resulted in coefficients of variatio n less than 12.15%. It was proven that both extraction procedures were similar. All performance criteria described in EPA Method 1613 and European Community legislations (2012/252/EU; 2011/1259/EU) were achieved. The method was applied as part of the Brazilian National Residue and Contaminants Control Plan (PNCRC). 95 samples of different species were collected from producers from different parts of the country and analyzed over a 12 month period. In 21 % of these samples, values higher than the LOQ were found. The highest contaminated sample presented an upperbound value of 0.39 pg WHO 2005-PCDD/F-TEQ/g, fairly lower than the adopted maximum level of these contaminants in fish, 3.5 WHO2005-PCDD/F-TEQ/g.

Keywords: *Dioxins, PCDD, HRMS, fish, validation*

Acknowledgement: *Conselho Nacional de Desenvolvimento Cientıfico e Tecnológico (CNPq)*

F-6
SAME SEPARATION WITH HALF THE COLUMN: EXTENDING THE LIFETIME OF YOUR GC COLUMN WITH COLUMN TRIMMING MAINTENANCE AND METHOD TRANSLATION

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Polybrominated diphenyl ethers (PBDEs) have been found to be persistent and bioaccumulative in the environment and are a concern for food contamination. The technical mixtures containing penta and octa congeners were voluntarily withdrawn in the United States in 2005, and the last remaining PBDE mixture, decaBDE, should be completely phased out by the end of 2013. While these mixtures have been phased out of production and use, the concentrations in the environment have not been declining and are currently still widely monitored. The analysis of PBDEs is challenging due to structural isomers that need to be chromatographically separated and thermally labile compounds of interest that may breakdown during gas chromatography. PBDEs included in EPA Method 1614 are well resolved on a 15 m × 0.25 mm × 0.10 µm Rtx®-1614 GC column, a 5% diphenyl, 95% dimethyl polysiloxane–type phase that was specifically designed to meet method resolution requirements. Using a short, thin-film column also allows the elution of decabromodiphenyl ether (BDE-209) without on-column thermal degradation. Monitoring efforts of the levels of PBDEs include a wide array of biota and environmental matrices. Non-volatile material may still persist even in cleaned-up final extracts, requiring GC column and inlet maintenance to be performed. Using a 15 m × 0.25 mm × 0.10 µm column, how many loops of the GC column can one clip for maintenance before the Method 1614 resolution requirements of BDE 49 and BDE 71 can no longer be met? The resolution between BDE 49 and 71 must be less than 40% valley height to meet method criteria.

Keywords: *Polybrominated diphenyl ethers, gas chromatography, method translation*

F-7
SYNTHESIS OF PAH-TETROL METABOLITES

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The first PAH to be identified as a carcinogen in tobacco smoke was benzo[a]pyrene. Benzo[a]pyrene diol epoxide is an extremely carcinogenic metabolite of benzo[a]pyrene. The presence of the epoxide in the bay region has been found to be a requirement for high activity diol epoxides derived from diol epoxides. The diol epoxides metabolizes further to PAH tetrols. In this paper we present the preparation of 8 PAH-tetraol isomers from benzo[a]anthracene diol epoxide and chrysene diol epoxides. Chromatographic and spectral behavior will be presented. This work was carried out in collaboration with CDC, USA.

Keywords: *PAH, tetrols, metabolites, synthesis, LC-analysis*

Acknowledgement: *Zheng Li and Andreas Sjodin, CDC, USA*

F-8
EVALUATION OF THE CADMIUM DISTRIBUTION AND POTENTIAL RISK IN THE CONSUMPTION ON THE WHEAT GRAINS PRODUCED IN BRAZIL

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The Brazilian National Residues and Contaminants Control Plan on Plant Products – PNCRC/Plant of the Ministry of Agriculture, Livestock and Supply (MAPA) is responsible for ensuring the food safety of plant origin products. Cadmium is one of the inorganic contaminants controlled in different plant species. The National Laboratory (Lanagro-SP) performed an exploratory study to evaluate the cadmium distribution profile in wheat produced in Brazil and its potential risk for consumers. The cadmium absorption in a plant species depends on several factors such as the soil pH, climatic conditions and soil type, and varies greatly between cultures and even within species. The species that represent 90% of the wheat grown in the world are: Triticum compactum (cookies and cakes), Triticum durum (dry pasta) and Triticum aestivum (bread). The latter is the most cultivated species in Brazil, with annual production ranging between five and six million tons, while domestic consumption is estimated at 10 million tons. After studies (2009) by the European Food Safety Authority (European Food Safety Authority – EFSA) the EU suggested a reduction in the currently cadmium maximum limits (0.2 mg kg^{-1}) for some wheat species. The new values would be 0.1 mg kg^{-1} for T. aestivum and 0.15 mg kg^{-1} for T. durum. The possibility to modify the maximum levels resulted in several discussions among countries, especially in cultivars with high Cd absorption. For example, according to the Canadian Grain Comission 78% of the T. durum samples exceed the proposed limit. For the exploratory study in Brazilian wheat samples, Lanagro-SP laboratory, following national and international regulations, developed a method for cadmium determination in vegetable matrices by Graphite Furnace Atomic Absorption Spectrometry (GF AAS). After the method validation, 31 wheat samples coming from the largest producing region were analyzed and all had concentrations smaller than the maximum limit allowed in current international law (0.2 mg kg^{-1}). Moreover, the data shows that Brazilian wheat presents reduced potential risk and, also, that if the maximum limit reduction proposed were approved, Brazil would not have commercial. Even with a limit of 0.1 mg kg^{-1}, all wheat samples analyzed in the exploratory program were in accordance with the new standard, suggesting the suitability of wheat grain produced nationally. Thus, providing the official laboratories with information concerning the factors affecting the cadmium absorption is essential in evaluating the non-complaint results during the future monitoring plan under the PNCRC/Plant. Another issue to be evaluated is the food safety of imported wheat, since almost half this product consumed domestically comes from other countries. Among the imported species, the T. durum should be controlled more strictly, because it is the one that presents the highest rates of cadmium bioaccumulation.

Keywords: *Wheat, inorganic contaminants, food control*

Acknowledgement: *This research was supported by CNPq and Ministry of Agriculture, Livestock and Food Supply – MAPA*

F-9
VALIDATION OF FAST METHOD FOR CADMIUM DETERMINATION IN MEAT SAMPLES SOLUBIZED WITH TMAH

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The validation of analytical methods aims to testify that it fits to purpose through experimental evidences. This is especially important for methods designed to monitoring and control of inorganic contaminants at trace levels in food products, considering health and commercial reasons. Cadmium is among the toxic substances controlled in muscle and edible offal of bovine animals, swine, poultry, horses, ovine and caprine, by Ministry of Agriculture, Livestock and Food Supply (MAPA), in Brazil. The proposed method for the determination of cadmium in kidney and liver of those species involves the solubilization, at room temperature, of 500 mg meat sample with 0.5 mL tetramethylammonium hydroxide (TMAH) 25% (*w/v*), after previous hydration, requiring less than 10 minutes for the sample preparation. The suspensions obtained are analyzed by Graphite Furnace Atomic Absorption Spectrometry (GF AAS) using $Pd+Mg(NO_3)_2$ as chemical modifier. The method was validated following procedures and acceptability criteria according to food international and national regulations, thus the validation was carry out at 200 and 1000 $\mu g\ kg^{-1}$ levels, corresponding to 0.2 and 1.0 of maximum level (ML). Poultry kidney samples were employed due to its low cadmium content. The detection and quantification limits were about 0.9 and 3.0 $\mu g\ kg^{-1}$, respectively and the method showed no matrix effect. The linearity was confirmed through graphics and statistical methods, and normal, homogeneous and independents residues were verified. Precision was evaluated as repeatability and intralaboratory reproducibility providing HorRat values < 0.5, indicating a precise method. The accuracy of the method was verified with a freeze-dried bovine liver reference material and recovery of 100 ± 7% (n=7) was obtained. Adopting the Youden Test, the method presented robustness for sample moisture (fresh or lyophilized), sample mass (500 or 300 mg), balance (analytical or semi), agitation type (vortex or manual), time between steps (4 or 10 minutes) and volumetric capacity tube (15.0 or 50.0 mL). The method scope extent has been carried out for kidney and liver of bovine animals, swine, horses, ovine and caprine. The developed and validated method allows fast and reliable determination of cadmium content in meat samples, being suitable for the National Plan for Control of Residues and Contaminants on Meat Products of the MAPA, in Brazil.

Keywords: *Food, inorganic contaminants, suspension, rapid methods, GF AAS, validation*

Acknowledgement: *This research was supported by CNPq and Ministry of Agriculture, Livestock and Food Supply – MAPA*

F-10
EVALUATION OF NEW ZIRCONIA-BASED CLEANUP SORBENTS IN THE DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN FATTY FOODS AND BEVERAGES

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Persistent organic pollutants (POPs) are compounds that are not easily degraded by chemical, biological, or photolytic processes. Therefore, they persist in the environment for long periods of time. POPs encompass harmful man-made compound classes such as dioxins, polychlorinated biphenyls (PCBs), chlorinated pesticides, and polyaromatic hydrocarbons (PAHs). Airborne transport of POPs may deposit them on fruits and vegetables, in waterways, or on crops that livestock consume. Because POPs often survive food processing steps, they are known to contaminate our food supply. The concept of dispersive solid phase extraction (dSPE), otherwise known as the QuEChERS method, continues to gain acceptance for the analysis of contaminants in food samples. The current sorbents for doing sample cleanup by QuEChERS include: C18 for the removal of lipid components, primary-secondary amine (PSA) for the removal of acids, polar pigments and sugars, and graphitized carbon black (GCB) for the removal of pigments such as chlorophyll. Cleanup of extracts from fatty foods presents a special challenge, as fat gets co-extracted with the target compounds. The cleanup commonly used for these types of samples is PSA/C18. However, PSA can introduce contaminants which may interfere in GC analysis, and C18 can reduce recovery of very hydrophobic compounds. Here we introduce two new sorbents for use in QuEChERS, Z-Sep and Z-Sep+, and compare them to PSA/C18 for cleanup of fat containing samples. Specifically, we will evaluate them in the analysis of PAHs and PCBs. These compounds are common in the environment as a result of various natural and man-made processes, and have found their way into the food supply through various means. The first application will demonstrate the analysis of PAHs in raw salmon by GC–MS and the second application will demonstrate the analysis of PCBs in whole cow milk by GC–ECD. For both applications, it was found that Z-Sep produced the best recoveries and lowest background of the cleanu p sorbents evaluated.

Keywords: *POP, SPE, extraction, fats*

F-11
QUATERNARY AMMONIUM COMPOUNDS (QAC) IN FOODSTUFFS – CURRENT SITUATION AND ANALYTIC METHOD

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H. Drinda, A. Romanotto, D. Wojciechowski; Eurofins SOFIA GmbH, Rudower Chaussee 29, 12489 Berlin Quaternary ammonium compounds (QAC) in foodstuffs – current situation and analytic method Quaternary ammonium compounds (QACs) are surface-active substances used as detergents and disinfectants acting against cell lipid walls in bacteria, for example benzalkonium chloride (BAC). Due to their activity against gram positive bacteria and some other microorganisms, QACs are also used as ingredients in human and veterinary medicinal products. Furthermore QACs are used as biocides, pesticides and additives for technical applications as well as in cosmetic products. Didecyl dimethyl ammonium chloride (DDAC–C10) is an example of the use of QAC as a pesticide. In the EU it is authorized as a plant protection product in ornamental crops and as biocide for disinfection. Because DDAC is authorized for these purposes, it´s present in food was unexpected. No specific MRL exist, only the default MRL of 0,01 mg/kg. But in conclusion, QACs are used in many products and therefore they are one of the major contaminants in the environment. Cases of contamination with QACs have been found since 2009 in milk products, 2011 and 2012 in fruits and high amounts in fresh herbs treated with a plant strengthener that contained DDAC. Thus QAC contamination in food has become a problem. In July 2012 the Standing Committee of the Food Chain and Animal Health (SCoFCAH) adopted guidelines for BAC and DDAC with guide values of 0.5 mg/kg (sum of DDAC respectively sum of BAC). The relevant QAC compounds in foodstuffs include DDAC (C10 to C12) and BAC (C10 to C18), other single QACs like benzyltrimethylammonium chloride (bromide), cetylpyridinium chloride, methyldodecyltrimethylammonium chloride as well as a further group QACs like alkyltrimethylammonium chloride (ATAC: C12, C14, C16) and DDAC with C14/C16/C18 alkylgroups. Our analytic method includes all these compounds. Sample preparation is based on liquid extraction and differs between samples with high and low fat content. Identification and quantification is performed by LC-MS/MS. Monitoring studies e.g. those of German fruit and vegetable authority QS between July 2012 and January 2013 still shows contamination with QACs. Altogether we analyzed about 3000 samples from the milk industry. DDAC as well as BAC are present in about 50 percent of the analyzed milk products. This is also true of the meat products. In the meat industry QACs are also the most popular cleaning agents. About a half of the measured meat samples contained DDAC and/or BAC. Therefore today it is very important to have fast and progressive analytic methods to control our food and to protect the consumer against environmentally contaminated foodstuffs.

Keywords: *Food, quaternary ammonium compounds, contaminants, LC–MS/MS*

F-12
ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BYPHENYL RESIDUES IN WILD ABRAMIS BRAMA AND CARASSIUS GIBELIO FROM THE DANUBE RIVER (SERBIA)

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The aim of this research was to evaluate the levels of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in Common bream (Abramis brama) and Gibel carp (Carassius gibelio). Fish was collected from the Danube River, at two different locations (Smederevo–L1 and Djerdap–L2), downstream from the Belgrade, in April 2013. The analysed compounds were: 16 OCPs (α-Hexachlorocyclohexane (α-HCH), β-Hexachlorocyclohexane (β-HCH), Hexachlorobenzene (HCB), γ-Hexachlorocyclohexane (γ-HCH), δ-Hexachlorocyclohexane (δ-HCH), Heptachlor, Aldrin, cis-Heptachloroepoxide (cis-HCE), trans-Heptachloroepoxide (trans-HCE), γ-Chlordane, α-Chlordane, *p,p'*-Dichlorodiphenyldichloroethylene (*p,p'*-DDE), Dieldrin, Endrin, p,p'-Dichlorodiphenyldichloroethane (*p,p'*-DDD), p,p'-Dichlorodiphenyltrichloroethane (p,p'-DDT)) and 7 PCB congeners (28, 52, 101, 138, 153, 180, 118). Determination of OCPs and PCBs were performed by a GC–ECD method. Student's paired t – test ($p<0.05$) for comparison of the mean values of OCPs and PCBs content data was performed using Microsoft Office Excel 2007. Cis-HCE, trans-HCE, *p,p'*-DDE, Dieldrin and *p,p'*-DDD were detected in Common bream from both locations. HCB was detected in fish samples collected from location 1 only. The amount of these pesticides were around, or bellow 1 ng/g fish, except for *p,p'*-DDE and p,p'-DDD. The quantities of the other tested pesticides were bellow the limit of detection, LD (LD=0.001 μg/g fat). Common bream from the both locations contained the highest amounts of p,p'-DDE (6.34±1.11 ng/g fish – L1; 9.23±0.93 ng/g fish – L2). PCBs (sum of congeners 28, 52, 101, 118, 138, 153, 180) and DDTs (sum of p,p'-DDE, *p,p'*-DDD and *p,p'*-DDT) in Common bream from location 2 (PCBs: 32.20±4.61 ng/g fish; DDTs: 14.52±1.41 ng/g fish) were significantly ($p<0.05$) higher than in the fish from location 1 (PCBs: 20.46±5.89 ng/g fish; DDTs: 9.59±1.98 ng/g fish).

HCB, cis-HCE, *p,p'*-DDE and *p,p'*-DDD were detected in Gibel carp from both locations. The other tested pesticides were bellow the LD. *p,p'*-DDE showed the highest content among the analysed pesticides (6.09±0.92 ng/g fish – L1; 2.40±0.79 ng/g fish – L2). The contents of the all detected pesticides, except of *p,p'*-DDD, were significantly lower in fish from location 2. The total PCBs were significantly lower in Gibel carp from location 2 than from location 1 (PCB-L1: 13.06±3.33 ng/g fish; PCB-L2: 31.50±3.15 ng/g fish), as well.

The content of the tested lipophilic compounds in fish species might be a consequence of the significant differences in their fat content (Common bream: 4.09±0.08%-L1, 6.46±0.15%-L2; Gibel carp: 3.58±0.09%-L1, 1.25±0.03%-L2). Based on the data obtained for contamination of fish with OCPs and PCBs, it can be concluded that there is no significant difference in their quantities on the tested spots on the Danube River.

Keywords: *River fish, organochlorine pesticides, polychlorinated biphenyls*
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F-13
ANALYSIS OF AS, CD, CU, FE AND PB IN CHOCOLATE AND COCOA PRODUCTS BY ICP–MS

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Chocolate is a processed, typically sweetened food produced from the seed of the tropical Theobroma cacao tree. Chocolate are consumed from Mayans period to present days. Cocoa plant is the basic ingredient in production of chocolate. In this study, we analized different tipes of chocolate, cocoa powder, cocoa mass and cocoa butter by ICP/MS.
The analysis was carried out in accordance with method BS EN 15763. Samples were prepared by microwave digestion (Multiwave 3000 by Anton Paar) and analyzed by Inductively Coupled Plasma–Mass Spectrometry (NEXIon 300X by Perkin Elmer).
In SP Laboratory, during 2013, we analyzed the 68 samples of chocolate (38 samples of chocolate produced in Serbia and 30 samples from EU), 30 samples of cocoa powder, 27 samples of cocoa mass and 18 samples of cocoa butter. In chocolate we determined As, Cd, Cu and Pb and results were in range: As 0.011–0.032 mg/kg, Cd 0.011–0.198 mg/kg, Cu 0.137-9.935mg/kg and Pb 0.011–0.141 mg/kg. In cocoa powder we determined the same elements and results were in range: As <0.001(LOQ)–0.094 mg/kg, Cd 0.012-0.256 mg/kg, Cu 4.998-49.94mg/kg and Pb 0.023-0.414 mg/kg, also in cocoa mass: As <0.001–0.087 mg/kg, Cd 0.015–0.607 mg/kg, Cu 8.686–29.84 mg/kg and Pb 0.031–1.260mg/kg, In cocoa butter we determined As, Cu, Fe and Pb and results were in range: As <0.001–0.033 mg/kg, Cu 0.017–0.398 mg/kg, Fe 0.388–1.994 mg/kg and Pb <0.001-0.091 mg/kg.
All analized samples were in accordance with Serbian and EU legislation.

Keywords: *Chocolate, cocoa products, ICP/MS*

F-14
ELABORATION OF THE GC–HRMS METHOD FOR SIMULTANEOUS DETERMINATION OF POLYBROMINATED, POLYCHLORINATED, MIXED POLYBROMINATED/CHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS, POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN FISH SAMPLES

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Established and comprehensively validated methodology for the analysis of five groups of persistent organic pollutants (POPs), including polybrominated, polychlorinated and mixed brominated-chlorinated dibenzo-p-dioxins and dibenzofurans (PBDD/DFs, PCDD/DFs and PXDD/DFs, respectively), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), in fish samples is presented. The method achieves the analytical standard of the EU Commission Regulation No 252/2012 that is used to determine the compliance of food products to maximum permissible levels of PCDD/DFs and PCBs. The presented analytical approach was based on well established and robust method of gas chromatography coupled to high resolution mass spectrometry (GC–HRMS) used for determination of PCDD/DFs and PCBs, which was extended to include PBDEs, as well as poorly investigated PBDD/DFs and PXDD/DFs at toxicologically significant levels. Intensive clean-up and fractionation procedures in combination with optimized instrumental parameters provided reliable detection and quantification of these compounds. The application of $^{13}C_{12}$-labeled surrogates of analyzed compounds allowed the internal standardization and accurate measurement of selected contaminants. The limits of detection within the selected POP groups achieved by this methodology ranged from 0.02 to 2.17 pg g^{-1} fat for fish. Method recovery, intraday and interday precision were calculated for fish oil spiked with native compounds and were in ranges 75–123%, 1.6–23% and 2.1–24% respectively. Recoveries of $^{13}C_{12}$-labeled standards ranged from 60% to 110% with exception of $^{13}C_{12}$-labeled octa-brominated and octa-chlorinated dibenzo-p-dioxins and furans and octa- through deca-brominated diphenyl ethers, for which typical recoveries were in the range of 30–50%. The developed procedure enables the fractionation of a range of toxic brominated and chlorinated pollutants present in a sing le fish sample and was used to measure the occurrence of priority POP groups in Baltic wild salmon. The analyses revealed the presence of poorly investigated PBDD/DFs and PXDD/DFs in salmon samples, with the latter occurring to a lesser extent, followed by PBDFs.

Keywords: *POPs, PBDD/DFs, PXDD/DFs, Fish, Simultaneous determination*

F-15
ACID DIGESTION IN CLOSED SINGLE USE PP TUBES. CAN IT BE USED FOR AAS OF SEAWEEDS?

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The use of seaweeds (macroalgae) both for human consumption and for technical applications, like biodiesel, has increased over the last years. In order to ensure both the safety and the quality of theseproducts, precise knowledge of the trace-metals content in the seaweeds are of key interest. The main challenge in these analyses is the digestion or mineralization step in which the trace elements are liberated from the samples. This is most commonly performed by microwave assisted acid digestion. Recently an interesting alternative using single use pp tube was published (1) but was only applied on plant sampled and analyzed by ICP-OES. Preliminary data indicates that this technique can also be applied to seaweeds and seaweed-based food products and that the digested samples were found suitable for analysis by atomic absorption spectroscopy (AAS), both as flame and graphite furnace AAS. (1) Matthew S. Wheal, Teresa O. Fowles and Lyndon T. Palmer, "A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP–OES) analysis of plant essential elements" Anal. Methods, 2011, 3, 2854

Keywords: *Seaweeds AAS, trace metal, sample digestion*

Acknowledgement: *The Lundbeck foundation and the Carlsberg foundation*

F-16
IMPACT OF COOKING CONDITIONS AND FAT CONTENT ON THE BIOACCESSIBILITY OF PCBs, PCDDs AND PCDFs IN MEAT PRODUCTS

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Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are persistent organic pollutants (POPs) causing harmful effects to human health. Food intake is the major pathway for human exposure to these substances. When quantifying ingestion exposure to toxic compounds, it is assumed that 100% of the contaminant is available for absorption into systemic circulation. However, only the bioaccessible fraction of this contaminant is mobilized from the contaminated food matrix into the digestive tract during digestion and induces toxic effects. The determination of the bioaccessibility of organic pollutants in food has to be considered for effective human exposure estimation and health risk assessment. In order to study food products "such as consumed", ie after cooking for the meat, the present study investigates the impact of cooking conditions and fat content on the bioaccessibility of the 209 congeners of PCBs and the 17 toxic congeners of PCDD/Fs in meat products. An in vitro gastrointestinal static method mimicking human physiological conditions was developed to assess the digestive bioaccessibility of these contaminants spiked in animal-derived food products. This artificial digestion system was coupled to an analytical method based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC–TOF/MS). In a first step, a GC×GC–TOF/MS-based method was developed for the multiresidue determination of 209 PCBs and 17 toxic PCDD/Fs in bioaccessible fraction in a single short time analysis (74 min). The performances of this method were validated in terms of resolution, linearity and limit of detection. In a second step, the feasibility of coupling the GC×GC–TOF/MS method with the in vitro digestion for the measurement of contaminant bioaccessibility was confirmed with the determination of the recovery rate after digestion of a crud e mix of contaminants. In a third step, this analytical set-up enabled to assess the impact of cooking process and matrix composition, especially lipid content, on pollutant bioaccessibility in meat products. The approach developed in this work enabled to identify contributing factors on PCB and PCDD/F bioaccessibility in cooked meat.

Keywords: *Bioaccessibility, PCBs, PCDD/Fs, meat products, GC×GC–TOF/MS*

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F-17
THE APPLICATION OF REPORTER GENE ASSAYS FOR THE DETECTION OF ENDOCRINE DISRUPTORS IN MILK

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Endocrine disruptors (EDs) are compounds known to interfere with the endocrine system by disturbing the action or pathways of natural hormones. Consequently, they may cause adverse health effects such as early puberty, urogenital tract abnormalities, infertility or cancer. Our diet is considered to be one of the main exposure routes to EDs. The food chain may be contaminated with natural EDs (such as phytoestrogens and mycotoxins) or synthetic EDs that can 'leach' from consumer products or landfill waste and find their way into the environment where many of them can bioaccumulate in biota. Additionally, processing activities and food contact materials may add further to the already existing pool of contaminants. The multitude of potential contaminants may be at safe levels individually but their mixture can create the "cocktail effect". Milk and dairy products are main components of our diet and therefore should be monitored for ED contamination. However, most assays developed to date utilise targeted chemical, chromatography based methods. Even though they provide an excellent tool for qualitative and quantitative analysis they lack the information about the possible biological activity of the monitored compounds and their mixture effects. This study utilises biological reporter gene assays (RGAs) to assess the total estrogen and androgen hormonal load in bovine milk. Analytes were extracted by liquid-liquid extraction with acetonitrile followed by clean up on a HLB column and the extract analysis by estrogen and androgen RGAs. Additionally, HPLC fractionation was used to facilitate the separation of endogenous hormones from other EDs, allowing the investigation of the input of each group into the milk's total hormonal load. The method has been validated according to EC decision 657/2002 as a quantitative screening method with CCβ enabling detection of low ppt levels of estrogen hormonal activity in milk.

Keywords: *Endocrine disrupting contaminants, reporter gene assays*

F-18
DETERMINATION OF ENDOCRINE DISRUPTORS IN BOVINE MILK USING ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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An endocrine disruptor (ED) is an exogenous agent that interferes with the body's endocrine system. The effects of exposure to EDs, depending on the time of the exposure, include urogenital tract abnormalities, reduced reproductive function, poor pregnancy outcomes, infertility or even cancer. EDs are a vast group of chemicals which consist of compounds of natural origin such as phytoestrogens or mycotoxins but also a wide range of man-made chemicals. Synthetic compounds may find their way into the food chain where a number of them can bioaccumulate. Additionally, processing activities and food contact materials may add further to the already existing pool of contaminants. Thus, our diet is considered to be one of the main exposure routes to EDs. Some precautionary legislation has already been introduced to control production and/or application of some persistent organic pollutants with ED characteristics. However, newly emerging EDs with bioaccumulative properties have recently been reported to appear at lower tiers of the food chain but have not been monitored at the grander scale. Milk and dairy products are a major component of our diet and a good indicator model for environmental contamination. Consequently it is of interest to monitor this matrix for EDs. However, most methods developed to date are devoted to one group of compounds at a time. The method described here allows simultaneous extraction, detection, quantitation and confirmation of more than 15 EDs in bovine milk (including phytoestrogens, mycotoxins, antimicrobials, UV-filters and pyrethroid metabolites). Analytes are extracted by liquid-liquid extraction with acetonitrile, followed by clean up on a HLB column and analysis employing a Waters Acquity UHPLC instrument with Waters Quattro Premier ^{XE} triple quadrupole detector operating in both positive and negative ESI modes. The method has been validated according to EC decision 657/2002. Established CCα and CCβ values facilitate fast, reliable, quantitative and confirmatory analysis of low ppb levels of a range of EDs in milk.

Keywords: *Endocrine disrupting contaminants, mass spectrometry*

F-19
THE TOTAL PCB-TASK: A COMPREHENSIVE HRGC–HRMS METHOD FOR ANALYSIS OF ALL 209 PCB CONGENERS IN FISH MATRICES

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Polychlorinated Biphenyls (PCB) have been known as being toxicologically relevant for a long period of time. There are different toxicological action modes of PCB, comprising dioxin-like (dl) and non-dioxin-like (ndl) activities, depending on specific chlorination patterns of the respective congeners. Derived from these facts, there have been ongoing discussions and activities in order to minimize human PCB exposure as far as possible and to regulate maximal allowed levels and intakes. One of these activities resulted in the "safe harbour levels" for PCB, issued by the Californian government in legislative Proposition 65. This issued level describes a maximum daily intake considered as being safe for human health, expressed as "total PCB".This legislative demand resulted in a question about the PCB content of food additives, as e.g. otherwise healthy fish oil products. It therefore implies the ability of precisely analysing the "total PCB" content in terms of determining all 209 PCB congeners. There have been several different approaches for respective methods and calculation modes, reaching from quantification against technical PCB mixtures over fractionation of PCBs to complete generic templates as e.g. US-EPA method 1668. With all these approaches showing difficulties to different extents, we developed a completed approach using a specialised multistep-cleanup in combination with a modern HRGC column and HRMS detection, which enables a comprehensive analysis of all PCB congeners with around 180 peak separations. We present results for this method as it has been applied on biota and biota-related samples, e.g. fish oils from South American origin from different fish species. We are able to show patterns of all PCB congeners as well as congener groups, opening access to examine preferences in transport and enrichment as well as metabolic behavior.

Keywords: *209 PCB, total PCB, fish oil, biota*

F-20
THE INFLUENCE OF THE CHICKEN HEN BREEDING METHOD ON THE PRESENCE OF PCDD/F, PCB AND PBDES IN EGGS

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Dioxin levels in agricultural products might be affected by the presence of the local emission sources located near the farms. Those might involve the industrial as well as municipal emission sources. In addition urban areas are significantly stronger contaminated with dioxin-like compounds. Hens are mainly exposed on dioxins and related compounds via the ingestion of feed of plant origin. However ingestion of grass, earth worms, and soil particles might have a large share in the total intake. De Vries (2006) have noticed that free range hens make more use of the latter sources due to outdoor run what may lead to an elevated dioxin content of their eggs. The average concentrations of 6 indicator PCB and 13 PBDEs in eggs from hens bred in cages (n=20) were 475.2±252.5 pg g^{-1} and 111.1±75.8 pg g^{-1} of fat. The calculated average PCDD/F/PCBs (1998) TEQ value was 0.956±0.952 pg g^{-1} of fat. The average concentrations calculated for free range hen eggs (n=19) were as follows: 3952.2±10204.8 pg g-1, 164.5±161.8 pg g^{-1} and 2.656±3.908 pg TEQ g^{-1} of fat, respectively for 6 indicator PCBs, PBDEs and PCDD/F/PCBs TEQ. No such high dissimilarity was observed in case of the median calculated concentrations. The median concentrations were 509.0 / 469.2 pg g^{-1}, 99.4 / 74.7pg g^{-1} and 0.812 / 0.494 pg TEQ g^{-1} of fat, calculated for 6 indicator PCBs, 13 PBDEs and PCDD/F/PCBs TEQ respectively (free range/cages). Results of this study have proved that conventionally and free range eggs have similar contamination level with PCDD/F, PCB and PBDEs in the most cases. The higher mean concentration values calculated for the free range eggs is due to occurrence of highly contaminated samples showing also significantly modified congener pattern. High absolute concentration values and the changed congener profile indicate on the presence of different contamination sources other than the environmental background of those substances only. The majority of the samples both produced under free range and caged conditions have the congener profile and the absolute concentration values typical for the environmental background of the persistent organic pollutants studied.

Keywords: *Eggs, dioxins, free range, PBDE*

F-21
PREVALENCE AND ANTIBIOTIC RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM ELEMENTARY SCHOOL CHILDREN'S HANDS IN SOUTH KOREA

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Staphylococcus aureus is an opportunistic food poisoning pathogen and one of the major antibiotic resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA). Elementary school children have not completed their immune system and this can make it difficult to fight off foodborne pathogens such as MRSA and VRSA. A hand, especially child's hand, is one of the most important reservoirs for cross-contamination of pathogens. Therefore, it is necessary to be monitored the hygiene of elementary school children's hands. The purpose of this study is to investigate the prevalence of *Staphylococcus aureus* on elementary school children's hands and to evaluate the toxin genes profiling and antibiotic resistance patterns of Staphylococcus aureus isolated on children's hands. A total of 200 elementary students divided into hand washed group (100 students) and unwashed group (100 students) were tested. The isolation and identification of *Staphylococcus aureus* was conducted according to the Korean Food Code. The possession of enterotoxin genes (set a ~ set q) in *Staphylococcus aureus* isolates was detected using a commercial polymerase chain reaction kit according to the manufacturer's instructions. The Kirby-Bauer method was used to determinate the antibiotic resistance patterns of Staphylococcus aureus isolates using 15 kinds of different antibiotics. *Staphylococcus aureus* ATCC 25923 was used as reference strain. *Staphylococcus aureus* was detected in 78 students (39%) out of all students. Comparing the hand washed group and unwashed group, the detection rate of *Staphylococcus aureus* was not a statistically significant difference. The 42 out of 78 *Staphylococcus aureus* isolates (53.8%) possessed one or more enterotoxin genes such as set a ~ set q. The isolates were resistant to oxacillin (32%), erythromycin (35%) and vancomycin (14%) among 15 antibiotics tested in this study.

Keywords: *Staphylococcus aureus, MRSA, VRSA, Food poisoning, Hand*

F-22
EFFICIENT EXTRACTION AND ANALYSIS OF PAHS FROM OLIVE OIL USING A NEW DUAL-LAYER SPE CARTRIDGE

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Olive oil can become contaminated with polynuclear aromatic hydrocarbons (PAHs) through exposure of the olives to pollution in the environment. Concern over exposure to these compounds has resulted in European Union Commission Regulation No 835/2011. This regulation sets a maximum limit for PAHs in edible oils of 2 ng/g benzo[a]pyrene alone, and 10 ng/g total for the sum of benzo[a]pyrene plus three additional PAHs. Low level PAH analysis is commonly done by either GC-MS or HPLC-FLD. Oily/fatty samples present an analytical challenge due to the heavy matrix effects often encountered. In the case of GC-MS, fatty matrix can cause contamination of the GC inlet, column and detector. In the case of HPLC, matrix can build up on the column, resulting in loss of chromatographic efficiency and /or an increase in system backpressure. Various cleanup techniques exist for fatty samples, including gel permeation chromatography, liquid/liquid extraction, and solid phase extraction (SPE), and some can be time consuming and expensive. The technique chosen for cleanup often depends on the analytical technique, specific target analytes, and required detection levels. In this work, a new SPE cartridge containing two different sorbent layers was evaluated in the simultaneous extraction and cleanup of PAHs from olive oil. The layers consist of Florisil and a mix of Z-Sep/C18. Olive oil sample was loaded directly onto the SPE cartridge, followed by elution of the PAHs with acetonitrile while fatty matrix remained bound to the sorbents. The resulting extract was concentrated, and analyzed by both GC–MS and HPLC–FLD. The dual-layer SPE cartridge was evaluated with olive oil samples spiked with light and heavy PAHs, and found to yield recoveries of >70% and % RSD values

Keywords: *PAH, Solid Phase Extraction, Dual Layer, Olive Oil*

F-23
STUDYING THE ACCUMULATION AND DISTRIBUTION OF MN, CU, CD AND ZN BY SOME GEORGIAN FEED PLANTS

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If the concentration of some elements in the organism exceeds the admissible limits, normal functioning of the organism is disturbed. Toxic elements could get into the organism with foods containing high concentrations of those elements after their long-term consumption. Such foods could be edible plants, growing on the soil, polluted with toxic elements. From this standpoint it is topical to study the process of absorption and distribution of toxic elements by edible plants. This will enable us to assess the probable threat in case of toxic pollution. We studied the process of absorption and distribution of Cd, Mn, Zn and Cu by the edible plants growing in Georgia: Saffron, barley, wheat, basil. The abovementioned involves the determination of the concentration of these elements in different organs of plants (roots, stem, leaves) at different stages of their growth. The elements (Cd, Mn, Zn, Cu) were introduced once into the purified soil directly in the form of sa lt solutions: ($CuSO_4 \cdot 5H_2O$; $CdSO_4$; $ZnSO_4$; $MnSO_4 \cdot 5H_2O$ (each of them separately); We introduced 1.5 g of each element in 15 L container with the soil purified from impurities and performed its homogeneization. We sow the plants in these containers. We collected sample plants in due intervals (about ≈ 30 days), washed them and divided into separate organs (roots, stem, leaves) and dried. We weighed the dried samples, placed them in laboratory vessels, ashed them and dissolved in bidistillate till the volume of 10 ml. Then we analyzed the prepared samples for the concentration of abovementioned elements by atomic absorption spectrometry (Analyst-800). The measurement results showed that the assimilation of elements by the plants was intense for the first month of its growth. Then the concentration of toxic metals tended to decrease. This is true as for the whole plant, so for its stem, leaves and roots. The concentration of these elements was the highest in the roots of whe at (Zn – 800 μg/g) and the lowest – in the leaf of barley (Cd – 10 μg/g). The investigation performed, allowed us to determine the intensity of assimilation of Mn, Zn, Cd and Cu from the soil by saffron, wheat, barley and basil and in which organs of the plant assimilate most. The obtained results will allow us to take into consideration the risks associated with the consumption of edible plants from the areas polluted with such metals.

Keywords: *Heavy metals, accumulation, feed plants, Cd, Mn, Cu*

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F-24
INTEGRAL FOODS SAFETY DETERMINATION BY BIOLOGICAL METHODS

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Food is the main biochemical bridge between the environment and the human body. The eco-toxicological monitoring of food's condition researching by methods of analytical chemistry is impossible due to the huge number of toxic pollutants in food, and the complexity of detection with high cost of chemical analyzes. A 50–60 thousands of xenobiotics are circulating in the atmosphere and undergoing the various changes - oxidation, hydroxylation, hydrolysis, isomerization and more. These changes as a result forms amount of substances, which enters the foods, and the toxic effects of these substances to the body is impossible to predict in practice. The problem of determining the toxic effects of food components on the human body can be resolved by studying foods safety primarily by means of the biological evaluation methods. The possibility of biological methods usage as a food safety complex characteristic and objective toxicological evaluation was proved. The conditions for biotesting with various test systems and conditions for extraction of toxic substances from plant material were studied. Such results have been obtained for a juicy plant material for the first time. Biotesting methods studied for a test-cultures: *Stylonichia mytilus, Daphnia magna S., Colpoda steinii, Allium cepa L., Salmonella typhimureum* TA-98, *Salmonella typhimureum* TA-100. Studies conducted in food samples and in the model samples: in samples containing the pesticides (β-cyfluthrin, tilt, sevin, phosalone), in samples containing the heavy metal ions (Pb^{2+}, Cd^{2+}), and in the samples containing compositions of pesticides and heavy metals. The toxic effects have not only the chemical substances, that entered during growing, transportation, processing, storage in raw materials and products, but also the toxic effects have natural toxicants – waste products of microbial spoilage of raw materials and products. These studies were conducted using the test-systems Stylonichia mytilus, Daphnia magna S. for herbal products and food products contaminated with waste products of microorganisms of the *Penicillium, Colletotrichum, Aspergillus, Aureobasidium, Pullularia, Candida, Endomyces, Gloesporium, Trichothecium genera*. All test cultures was indicative to the toxic metals, pesticides and mycotoxins in the model experiments with monotoxins and the compositions of toxic substances. The indicators of overall toxicity are set for 94 kinds of foods from a raw materials, and using these test systems revealed dose-effect. Thus, the biological testing enables us to determine the overall toxicity of the research subjects with no information about the chemical nature of the toxic pollutant, and to predict the integral impact of toxic samples on living organisms. The biotesting offers significant advantages in the economic, methodological and ethical matters comparing with trials on animals.

Keywords: *Overall toxicity, test-cultures, Stylonichia mytilus*

F-25
16 EU PAH IN EDIBLE FATS BY STYRENE DIVINYLBENZENE SPE CLEAN-UP AND SOLVENT VENT PTV GC–MS ANALYSIS

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Regulation (EU) 835/2011 identifies benzo(a)pyrene (BaP), benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF) and chrysene (CHR) as indicators for the occurrence of the 16 EU priority Polycyclic Aromatic Hydrocarbons (PAH) in food, replacing the BaP (Regulation (EU) 1881/2006). Regulation (EU) 836/2011 defines specific performances criteria that analytical methods should have before being applied for the official control of PAH (i.e. LOQ and LOD should be ≤0.9 and ≤0.3 µg/kg respectively). PAH are highly lipophilic substances thus, in food analysis, their selective isolation from fats is a challenging issue. A fast, effective and sensitive method for the determination of all the 16-EU priority PAH in edible fats and oils was set up. Two grams of sample spiked with the deuterated analogues of the four indicator PAHs were dissolved in c-Hex and loaded on a styrene-divinylbenzene (SDB) SPE column (1 g/6mL): the fat was washed away with c-Hex and the analytes selectively eluted with ethyl acetate. After the SPE, upon solvent removal, the few milligrams of fat remained were re-dissolved in a DCM/c-Hex (1:1) mixture and further purified by size exclusion chromatography (small column: 1 cm diameter, 50 cm length). The gas-chromatographic separation of all the 16 PAH was achieved on a DB-17MS (20 m × 0.18 mm × 0.180 µm) capillary column in a 6890N Agilent Technologies GC coupled to a 5973inert single quadrupole mass spectrometer, using $^{2}H_{12}$Perylene as syringe standard. Large-volume injection performed in programmed-temperature-vaporization (LVI-PTV) shown to be the right approach to enhance the sensitivity of all the 16-PAH GC-MS analysis, particularly of the last eluting high molecular weights compounds (pyrenes) which have poor chromatographic responses with broadened peaks. Moreover LVI-PTV succeeded in reducing the signal discrimination among low and high molecular weight compounds. Optimization of the LVI–PTV injection parameters (injection volume, initial injector and oven temperatures, vent flow, vent time, and vent pressure) was performed following already published studies (1). Both the LVI-PTV technique and the use of 1g SDB stationary phase yielded to a substantial increase of the signal to noise ratio for all the sixteen analytes enabling the correct quantification of 0.9 µg/Kg (LOQ) also in fats and oils. The LOQ was tested with repeated analysis (n=7) on spiked fat obtaining good recoveries and reproducibilities. The performances of the developed method were assessed in terms of instrumental linearity, LOQ, LOD, intra-laboratory reproducibility, accuracy and measurement uncertainty for all the 16 EU priority PAHs.

Keywords: *Polycyclic Aromatic Hydrocarbons, GC–MS, Food, PTV, PS-DVB-SPE*

F-26
FROM GC–MS TO GC–MS/MS TRIPLE QUADRUPOLE ANALYSIS OF NDL PCBS IN FOOD: REDUCED CLEAN-UP AND INCREASED SENSITIVITY

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Commission Regulation (EU) 1259/2011 set for the first time maximum levels for non dioxin-like PCBs (NDL-PCB) in foodstuffs. Six PCB congeners (28, 52, 101, 138, 153 and 180) have been identified as indicators to assess the human exposure upon food consumption and their sum accounts of about half of the NDL-PCB contamination in food. Laboratories in charged of official control are requested to monitor these contaminants in food and feed and check the compliance to regulatory limits. The limits in force are not very strict because the too high LOQs of the EU laboratories yield, in the upper bound (u.b.) approach, to PCB-sums very close to the maximum levels even if no congener is quantified. Therefore is strongly recommended to enhance the sensitivity of the analytical methods because a revision of the maximum levels is foreseen in three years time. The procedure applied in our laboratory for the 6-indicator-PCBs is a fast and high trough-put method involving a pressurized ASE extraction of the fat and a clean-up on an Extrelut NT3 column acidified with sulfuric acid and sequentially connected on top of a 1 g/6mL silica SPE column. The sample is finally injected on a GPC-column to perform size exclusion purification. The chromatographic separation was achieved on a SGE-HT8 PCB capillary column (60 m × 0.25 mm × 0.25 µm) by a GC–MS single quadrupole using as internal standards PCB155 and PCB198. LOQs were 1 ng/g fat in all the matrices. Recently a new GC–MS/MS (7890A and 7000 Triple Quad, Agilent Technologies) was installed in our laboratory for the analysis of NDL-PCBs and therefore the possibility to speed up the method by reducing the purification steps and to lower the limit of quantification was essayed. Isotope dilution was also introduced adding to the sample the six ^{13}C –analogues of the indicator PCB at the beginning of the analysis. Before injection the ^{13}C-PCB155 was added to the sample as syringe standard. As a result of the enhanced selectivity and sensitivity of the triple quadrupole MS detector operated in MRM, no GPC clean-up was performed anymore and the LOQ was brought to 0.5 ng/g fat. The LOQ was tested with repeated analysis (n=7) on spiked samples of different matrices (milk, eggs, muscle) obtaining good recoveries and reproducibility. Specificity was assessed doing repeated analysis (n=7) of the same blank matrices. The revised method was also tested analysing again stored aliquots of samples dispatched from the European Reference Laboratory for Dioxins and PCBs in Feed and Food in the frame of European Proficiency Test exercises (2010: milk fat and pork fat; 2011: fish oil; 2012: lard, whole egg and yolk). The obtained results were in good agreement with the PTs consensus values: maximum deviation of 23% for the single congeners and of 18% for the u.b. sum of the six PCBs were obtained doing duplicate analysis of each of the six PT materials.

Keywords: *NDL-PCB, GC–MS/MS, food, isotopic dilution*

F-27
SELECTIVE AND SENSITIVE DETECTION AND QUANTIFICATION OF STOCKHOLM CONVENTION POPS, INCLUDING DIOXINS, USING ATMOSPHERIC PRESSURE GAS CHROMATOGRAPHY MS/MS

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In recent years, the UNEP program focused on the development of analytical capacity for the analysis of POPs concluded that it is very challenging for countries to analyse all compounds included in the global monitoring program. This is especially true for developing countries. Since both LC and GC methods are required, it is difficult to employ a universal technology to meet the testing requirements. Furthermore, dioxins, considered to be the most problematic POPs, are analyzed using high resolution GC/MS in order to achieve the necessary sensitivity and selectivity. Alternative technologies to enable the analysis of all POPs on the Stockholm convention should help to improve capabilities for detection and quantification of POPs. Atmospheric pressure GC (APGC), first developed in the 1970s, has recently made a comeback as an alternative to high resolution EI–GC/MS and EI–GC/MS/MS. For many of the POPs on the Stockholm Convention, only the molecular ion is formed under APGC ionization conditions. By avoiding fragmentation, the sensitivity in MRM or SIR mode is enhanced. This technique shows high potential for the selective and sensitive analysis of dioxins. In this work, APGC with a Xevo TQ-S tandem quadrupole mass spectrometer was used in MRM mode under "dry" N_2 conditions. Standards for dibenzo-p-dioxins and furans were obtained from Wellington Labs. Data were acquired with two transitions for each of the native compounds and their ^{13}C analogues. The results demonstrated that the sensitivity achieved using APGC–MS/MS is comparable with high resolution GC/MS, the standard for dioxin analysis. Injection of a 1/10 dilution of calibration standard CSL of concentration range 10–100 fg/µl resulted in S/N from 10 to 65. A calibration curve was generated by analyzing 1/10 CSL up to CS4 standard. Coefficients of determination (r^2) for all compounds over the calibration range were all > 0.998. To test the sensitivity of the system, the lowest calibration point was diluted 1/10. The S/N for the first or second MRM ranged from 10 (10 fg TCDD/TCDF) to over 50 (other congeners 50–100 fg). Repeatability for n=10 injections of the CSL standard were all less than 10% RSD. The relative standard deviations ranged from 2.5 to 9.6%. In addition, ion ratio deviations were all less than 20% across the calibration range. These are extremely good results and meet the specification often set for high resolution GC/MS systems. With the selectivity and sensitivity now achievable with APGC–MS/MS there is an alternative for the analysis of dioxins and other POPs on the Stockholm Convention.

Keywords: *POPs, Dioxins, APGC, MS/MS*

F-28
HUMAN DIETARY EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS: RESULTS OF THE SECOND FRENCH TOTAL DIET STUDY

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The Second French Total Diet Study (TDS 2) is a large scale study aiming at providing exposure assessment data of the French population to a wide range of chemical hazards through their diet. The sample collection was constituted in 2007–2008 on the basis of a previous consumption habits study (INCA2, 2006–2007). Two main criteria were considered: *(i)* the most heavily-consumed foods and *(ii)* foods less heavily consumed but likely to be highly contaminated. A total of 212 different food types were thus selected, covering around 88% of dietary consumption in the adult and child populations. Finally, 1319 composite samples, each one from 16 sub-samples, were prepared as consumed by the population. Among the selected substances, congeners of persistent organic pollutants and assimilated were analysed at LABERCA (Oniris, Nantes, France) including 17 polychlorodibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), 12 "dioxin-like" and 6 "non-dioxin-l ike" polychlorinated biphenyls (dl-PCBs, ndl-PCBs), 16 perfluorinated alkyl acids (PFAAs), 8 polybrominated diphenyl ethers (PBDEs), 3 polybrominated biphenyls (PBBs), 3 hexabromocyclododecane enantiomer pairs (HBCDDs) and 20 polycyclic aromatic hydrocarbons (PAHs). Analytical methods were based on high resolution or tandem mass spectrometry couplings (GC–HRMS, LC–MS/MS or GC–MS/MS), with isotopic dilution approach for quantification (ISO 17025 standard). The results show a significant 4 fold decrease in exposure to PCDD/Fs and PCBs in the French population compared to the previous 2005 and 2007 available assessments. This trend is consistent with the global decrease observed in Europe. However, a small but significant proportion of consumers.

Keywords: *Total Diet Study, PCDD/F, PCB, BFR, PFAA*

F-29
EFFECTS OF FOOD QUALITY ON HEAVY METALS CONTENT IN HAIR OF RESIDENTS OF CITIES IN GEORGIA

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The purpose of the research was to apply the chemometric techniques for determination of the relationships between the level of environmental contamination, food quality and content of heavy metals (copper, lead, cadmium, zinc) in the scalp hair of the inhabitants of some cities of Georgia (Tbilisi, Rustavi, Mtskheta, Kutaisi, Batumi and Zestafoni). Studies were carried out using differential pulse polarography and roentgen-fluorescence spectroscopy (ElvaX). Sample collection and preparation were carried out using standard procedures. It is noteworthy that for all of the inspected subjects (about 500 human hair samples were analyzed), the contents of cadmium and copper were in the normal range and the high level of zinc was observed only in individual cases. A comparison of averages deviation from the norm of a microelement in the hair of the inhabitants of the central and peripheral areas of the city of Tbilisi, as well as living near a metallurgical plant in Rustavi, or away from it showed no significant difference, and at the same time, significant differences were observed for one the same quarter, but for members of the same family concentration ratios were the same. Lead has been identified as a highest risk public health problem among children. In Mtskheta results of the analysis of the hair of pupils under 14 years of age and instructors of the orphanage showed, that the pupils have elevated levels of lead ions – 7 μg/g (the WHO has established a guideline value for maximum lead levels - 5.0 μg/g), but the surrounding area did not exceed the permissible limit of lead content in the soil – 24.9 μg/dL (allowable upper limit is 32.0 μg/dL). When comparing the content of heavy metals in the hair of residents of Zestafoni and Batumi, we found, that in the hair of residents of the seaside resort Batumi the high content of lead is more common than among the inhabitants of the industrial Zestafoni. In all cities attentio n is drawn to the fact that the deviation from the norm of an element in most cases is typical for members of one family. All this allows us to suggest that, obviously, the deviations from the norm of heavy metals in human hair, to a greater extent depend on the diet and food. Therefore, in order to make a clear conclusion causes abnormal contents of a microelement in human hairs, is necessary to analyze the main agricultural products of the regions of Georgia, which supply the studied cities.

Keywords: *Heavy metals, hair, roentgen-fluorescence spectroscopy*

F-30
DETECTION OF FECAL CONTAMINATION ON BABY SPINACH LEAVES USING HYPERSPECTRAL FLUORESCENCE IMAGING AND PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS

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Foodborne illnesses caused by contaminated fresh leafy greens and their economical consequences are worldwide issues. Fecal contamination on fresh produce can contain pathogenic bacteria. Hyperspectral fluorescence images of baby spinach leaves (*Spinacia oleracea* L.) from 464 to 800 nm were taken; violet excitation was supplied by two LED-based line light sources at 405 nm. The adaxial and abaxial surfaces of the leaves were contaminated with fecal matter at six dilution levels and allowed to dry before imaging. Distilled water was used in dilutions. Partial least squares discriminant analysis coupled with masking detected 100% of fecal contamination up to 1:10 dilution. The technique detected 90, 70 and 63% of 1:20, 1:30 and 1:40 dilutions and 7% of leaves had false positives. Additional image processing procedures may be needed to enhance the detection rates for the higher dilution levels and to reduce the false positives. An on-line fecal contaminati on detection device for fresh leafy greens can increase food safety and protect consumers from related illnesses.

Keywords: *Hyperspectral Imaging, Fluorescence, Contaminant Detection, Baby Spinach Leaves*

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F-31
TOWARDS PROBLEMS IN THE DETERMINATION OF SHORT CHAIN PFAS BY UPLC–MS/MS IN FRUITS AND VEGETABLES

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PFCAs (perfluoroalkyl carboxylic acids) are broadly used surface chemicals found in food contact materials and textiles, however, for their toxicity and presence in humans they are under scrutiny. Moreover it was shown that an uptake from soil into food-plants occurs. In many cases analytical problems can be observed in the determination of short-chain PFCAs especially PFBA (perfluorobutanoic acid) in certain matrices of plant origin. PFBA is a polar PFCA and its retention on commonly used HPLC columns is low. Other polar compounds from the sample matrix may also be poorly retarded and may co-elute with PFBA. As ionization in UPLC-MS is a matter of competition between different species, the co-eluting compounds may influence the ionization of PFBA. The effect may be remarkable depending on the type and the concentrations of low retarded matrix constituents and may lead to a concern about the reliability of PFBA results obtained by conventional HPLC–MS/MS pro cedures. Here, we show the results of an investigation performed targeting the analytical reliability of the PFBA determination in extracts of different fruits and vegetables. High resolution mass spectrometry (HR–MS/MS) was applied and different chromatographic systems were successfully used to optimize the retention behaviour of the compounds involved.

Keywords: *PFCAs, UPLC–MS/MS, fruits, vegetables*

F-32
INORGANIC ARSENIC –SPE HG–AAS METHOD FOR RICE TESTED IN-HOUSE AND COLLABORATIVELY

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Arsenic (As) is a trace element present in the environment and consequently in various food items, e.g. rice, which may contain relatively high concentration of arsenic compared to other foodstuffs of plant origin. Rice contains most often three forms of arsenic; inorganic arsenic (iAs) and the methylated species monomethylarsonic acid (MA) and dimethylarsinic acid (DMA). Dietary intake of iAs is of special concern due to its carcinogenicity to humans, whereas DMA and MA are considered of less toxicological importance. Rice grains and rice-based products are staple foods in many countries and is one of the major contributors to the iAs exposure in many countries. The work presented here describes the development, validation and application of a simple and inexpensive method for inorganic arsenic (iAs) determination in rice samples. The separation of iAs from organoarsenic compounds (MA and DMA) was done by off-line solidphase extraction (SPE) followed by hydride generation atomic absorption spectrometry (HG–AAS) detection. Water bath heating (90°C, 60 min) of samples with dilute nitric acid and hydrogen peroxide solubilised and oxidized all iAs to arsenate (AsV). Loading of buffered sample extracts (pH 6±1) followed by selective elution of arsenate from a strong anion exchange SPE cartridge enabled the selective iAs quantification by HG–AAS, measuring total arsenic (As) in the SPE eluate. The in-house validation gave mean recoveries of 101–106% for spiked rice samples and in two reference samples. The limit of detection was 0.02 mg/kg, and repeatability and intra-laboratory reproducibility were less than 6 and 9%, respectively. The SPE HG-AAS method produced similar results compared to parallel high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (ICP-MS) analysis. The SPE separation step was tested collaboratively, where the laboratories (N=10) used either HG-AAS or ICPMS for iAs determination in a wholemeal rice powder. The trial gave satisfactory results (HorRat value of 1.6) and did not reveal significant difference (t test, p>0.05) between HG–AAS and ICP–MS quantification. The iAs concentration in 36 rice samples purchased on the Danish retail market varied (0.03–0.60 mg/kg), with the highest concentration found in a red rice sample.

Keywords: *Inorganic arsenic, speciation, SPE HG-AAS, validation, interlaboratory comparison*

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F-33
APPLICATION OF COLD VAPOR – ATOMIC ABSORPTION SPECTROPHOTOMETRY FOR HG ANALYSES OF FISH SAMPLES. VALIDATION OF THE METHOD

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This paper presents the results of the implementation and application of methods for Hg analysis in fish samples. The determination of Hg by Cold Vapor-Atomic Absorption Spectrophotometry was carried out using calibration curves, prepared from certified stock standard solutions of this element. A Certified Reference Material (DOLT-2) was employed as a quality control of the analysis. The values of Mercury were finding content below the permissible maximum limits established by WHO. For the validation was employed: "Guia harmonizada de validacion del metodo y estimacion del calculo de incertidumbre aplicado a la determinacion de contaminantes inorganicos" studied in the theoretical course of validation organized by the proyect RLA 5060.

Keywords: *Fish samples, heavy metals, AAS*

Acknowledgement: *The obtained results obtained are relationated with Proyect RLA 5060 and CUB 5018 and with the funding of OIEA and AENTA.*

F-34
USE OF HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY TO DETERMINE ARSENIC CONTENT IN FORAGE SAMPLES

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Arsenic has been considered for a long period to be an essential trace element for animal growth and development. However, it has recently been included in the group of the substances undesired in animal forages. Arsenic found in the composition of pesticides can lead to accumulation in soil and plants resulting traces of arsenic found in forages and food. The arsenic contamination of the animal foods can be minimized by a strict control of its concentration in the forages. The admitted level of arsenic in the forages was set by EC Directive 2008/76/EC which modified Appendix I to EC Directive 2002/32/EC of the European Parliament and European Council regarding the substances undesired in animal forages, published in the Official Journal of the European Union no. L 198 of 26 July 2008. The method of hydride generation atomic absorption spectrometry to determine arsenic content was used on the following bulk forage samples: alfalfa (greenchop, high-moisture h ay, low-moisture hay, high-moisture silage, low-moisture silage), corn silage and wheat silage. The samples were submitted to wet digestion, with microwaves, in a closed system and then turned into solutions using a mixture of HNO_3 and H_2O_2 (5:2), transferred into graduated flasks and aspired into the atomic absorption spectrometer using the hydride generator. Arsenic absorbance was measured at 193.7 nm wavelength. The method was validated "in house" determining the following parameters: accuracy; fidelity; repeatability; reproducibility; sensitiveness; detection limit; quantification and tracing limit, according to the requirements of SR EN ISO / CEI 17025:2005 standard. The trials required by the process of validation of this analytical method, the values of the parameters ranged within the admitted limits. The use of hydride generation atomic absorption spectrometry to determine the arsenic content of the forages produced the following arsenic concentrations: 0.22 ppm – greenchop alfalfa; 0.71 ppm - low-moisture alfalfa hay; 0.82 ppm – high-moisture alfalfa hay; 0.75 ppm – high-moisture alfalfa silage; 1.06 ppm – low-moisture alfalfa silage; 0.54 ppm – corn silage and 0.32 ppm – wheat silage. These values show that the arsenic contamination of the forages is very low, in agreement with the limits imposed by the acting laws (max. 4 ppm).

Keywords: *Arsenic, forages, contaminants, atomic absorption spectrometry*

F-35
DETERMINATION OF HEXABROMOCYCLO-DODECANE STEREOISOMERS IN FISH BY SUPRAMOLECULAR SOLVENT-BASED MICROEXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

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A simple and rapid method that combines a single-step sample treatment with chiral liquid chromatography coupled to tandem mass spectrometry was developed for the determination of hexabromocyclododecane (HBCD) stereoisomers in fish. HBCD is an industrial additive consisting of six stereoisomers [(+)-α-, (–)-α-, (+)-β-, (–)-β-, (+)-γ and (+)-γ-HBCD] that frequently contaminates natural waters and fishes living in them. The composition of HBCD in fish differs from that of commercial products because the HBCD stereoisomers are absorbed and metabolized at different rates. The most abundant isomers in industrial products and fishes are γ- and α-HBCD, respectively. Deviation of the enantiomeric ratios [ERs, defined as the molar ratio of (+)- to (–)-enantiomers] from their original value (i.e. ER=1 for racemic mixtures) has been also observed for the three isomers (α-, β- and γ-HBCD). In the proposed method, HBCD stereoisomers were extracted from fish samples with a supramolecular solvent (SUPRAS) made up of reverse aggregates of decanoic acid (DeA) and generated by adding water to a solution of the surfactant in tetrahydrofurane. The sample (750 mg) was vortex-shaken with 600 µL of SUPRAS for five minutes, the SUPRAS extract containing the analytes was separated from sample particles by ultracentrifugation and then diluted 1:1 with methanol before its analysis LC/QQQ-MS. Driving forces for the microextraction of HBCD in the SUPRAS involved both dispersion and dipole-dipole interactions. Separation of the HBCD stereoisomers was performed on a stationary phase of β-cyclodextrin and their quantification in a mass spectrometer equipped with an electrospray ionization source and a triple quadrupole mass analyzer. The extract was large enough to provide the required number of aliquots to apply the standard addition method for determining the target analytes using a single sample. Recoveries were independent of most of experimental parameters, so the method was very robust, and no clean-up steps were applied, that rendering the procedure very simple. Quantitation limits for the determination of HBCD stereoisomers in hake, cod, sole, panga, whiting and sea bass were within the intervals 0.5-5.6 ng g^{-1}, and recoveries for fish samples fortified at the ng g^{-1} level ranged between 87 and 114% with relative standard deviations from 1 to 10%.

Keywords: *Hexabromocyclododecane, fish, supramolecular solvents, chiral analysis, LC/MS/MS*

F-36
DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS, DIBENZOFURANS AND DIOXIN-LIKE BIPHENYLS IN FISH AND CANNED FISH AND INVESTIGATION OF THE CONGENER PROFILE AND SIMILARITY BETWEEN THE SAMPLES

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Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs) are highly persistent environmental pollutants. Significant contribution of human exposure to PCDDs, PCDFs, and DL-PCBs has been attributed to animal origin food intake. Fish are an important element for a human diet as indicated by the number of recommendations to increase the consumption of fish but they can also be considered as one of the most significant indicators for the impact of persistent organic pollutants such as dioxin (PCDDs/PCDFs) and DL-PCBs. According to the current WHO guidelines (Commission Regulation (EU) No 1259/2011 of 2 December 2011) for the determination of dioxin and PCBs in foodstuffs is required to mark the 17 most toxic congeners of PCDDs/PCDFs and 12 congeners of DL-PCBs. The content of these compounds expressed their total value known as toxic equivalent TEQ. Acceptable level of dioxin in fish is 3.5 pgWHO-TEQ/g fresh weight (f.w.) and the limit value of the sum of dioxin and DL-PCBs is 6.5 pg WHO-TEQ/g f.w. A total of 103 samples of marine fish, fresh, frozen and smoked and a canned fish were research material. Samples were homogenized, dried, extracted in Soxhlet apparatus and clan up by using membrane techniques and multilayer silica gel columns. A sensitive isotope dilution technique in gas chromatography coupled with tandem mass spectrometry ID-GC-MS/MS was applied for the determination of congeners of PCDDs/PCDFs and DL-PCBs. Internal standard of NK-LCS-G (Wellington Laboratories, Canada) was added before the extraction. The study indicates that the marine fish in some cases are contaminated with dioxin and DL-PCBs in amounts exceeding the permissible content. Statistical analysis includes congener profile, correlation study and cluster analysis was performed. Cluster analysis use as a data mining technique allowed the similarity of groups of objects and the display of objects based on the degree of integration within the same group. The objects were grouped according to the method of single bond. The Euclidean distance was the most effective among the analyzed distance measures based on its ability to distinguish between samples. Pearson correlation coefficients showed significant relationships between PCDDs, PCDFs and DL-PCBs in the fish. There is a suggestion that these contaminants have similar sources and/or bioaccumulation behavior in the marine food web.

Keywords: *Dioxin, PCBs, fish analysis, cluster analysis*

F-37
INVESTIGATION OF CONTENT OF HEXABROMOCYCLODODECANE ISOMERS IN MARINE FISH AND CLUSTERING OF SAMPLES

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Wide use of hexabromocyclododecane (HBCD) as an additive flame retardant in expanded (EPS) and extruded (XPS) polystyrene foams has led to widespread contamination and accumulation of this compound in many biotic and abiotic environmental compartments [1]. A sensitive ID-LC-MS/MS method was applied for the determination of α-, β- and γ-HBCD in fish tissue. Samples were homogenized, freeze-dried and extracted in a Soxhlet apparatus. Internal standard of d18-γ-HBCD was added before the extraction. Clean-up procedure was conducted using a membrane techniques and multilayer silica gel column. The isotopic dilution techniques (ID-LC–MS/MS) were used to determine of HBCD in selected marine fish. Chromatographic separation of the three α-, β-, γ-HBCD isomers was achieved. LC apparatus was equipped with Phenomenex Kinetex (C18; 50 × 2.1 mm) analytical column. Isocratic elution was used with a mobile phase of 30% of water and 70% of methanol. The three of HBCD isomers were detected by the quantitative SRM transition of the chlorine adduct [M-H+Cl]− (676.6 *m/z*) of the HBCD isomers to the quasi-molecular [M−H]− (640.6 *m/z*) ion and the confirmative SRM transition of the molecular ion to the bromine ion (81.1 *m/z*). ID–LC–MS/MS method is characterized by a low limit of detection in the range of 1 to 3 pg/g fresh weight, depending of the isomers and the recoveries for HBCD isomers between 89% and 124%. In the study, γ-HBCD levels were determined in all of the investigated samples. Statistical analysis was performed to determine the differences between the analyzed samples. Cluster analysis (CA) use as a data mining technique allowed the differentiation of groups of objects and the display of objects based on the degree of integration within the same group. The objects were grouped according to the method of single bond. The Euclidean distance was the most effective among the analyzed distance measures based on its ability to distinguish between samples. The analyzed fish samples were grouped into three clusters relative to the concentration of the HBCD isomers.

[1] Priority Existing Chemical Assessment Report No. 34. Hexabromocyclododecane. June 2012. Australia. ISBN 978-1-74241-715-8. Online ISBN: 978-1-74241-716-5. Publications approval number: D0755JUNE 2012.

Keywords: *Hexabromocyclododecane, fish analysis, LC–MS/MS, cluster analysis*

F-38
EPIDEMICS AND THE MICROBIOLOGICAL QUALITY OF CARROTS

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Typical sources of microbes that may cause food poisoning related to carrots are field (soil), irrigation water, wild animals, and insufficient storage and process hygiene after harvesting. Several microbes including pathogens have been detected from carrots in some international studies, although carrots have only rarely been implicated in food-borne epidemics. Reported epidemics associated with Yersinia pseudotuberculosis have mainly been from Finland. Internationally, *E. coli* has been identified as a source of a few carrot epidemics, and some other pathogens have also been implicated as probable causes of epidemics. In our present study the microbiological quality of carrots was examined. The highest aerobic plate counts were detected from washed, unpeeled carrots. *E. coli* was not detected. Higher amounts of coliform bacteria and enterobacteria were detected in carrots from the first steps in the processing line than in carrots from later phases of the p rocess. Y. pseudotuberculosis was not detected in the carrot samples by cultivation methods, but non-pathogenic Y. enterocolitica was detected from most carrot samples. A Real-Time PCR method identified pathogenic Y. enterocolitica in several carrot samples. However, no pathogenic Y. enterocolitica strains were found in cultivation of the positive samples, indicating that only low levels of pathogenic Y. enterocolitica were present in carrots.

Keywords: *Carrot, quality, epidemics, Yersinia*

F-39
MEASUREMENTS OF TRACE METAL CONCENTRATIONS IN THE LIVER OF SHEEP AND GOATS FROM NORTHERN GREECE, USING RADIOISOTOPE-EXCITED ENERGY-DISPERSIVE XRF SPECTROMETRY

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Knowledge of the levels of toxic metals in livestock is important for assessing the potential effects of pollutants on the animals themselves and in quantifying contaminant intake in humans. Although metal concentrations in sheep have been measured in many countries, there is a scarcity of data for Greece. The aim of the present study was to determine metal concentrations in the liver of sheep and goats from central Macedonia, northern Greece. A total of 54 animals were randomly selected between November 2011 and May 2013.

Liver samples were dry-ashed in a muffle furnace at 450°C. The residue was pulverised in an agate mortar and pressed into standard 1.27 cm-diameter pellets.

For the determination of the trace element concentrations, an energy-dispersive XRF spectrometry arrangement was employed. Photons emitted from an annular Cd-109 source were used for sample excitation, while XRF spectra were recorded using a Si(Li) detector. Spectral analysis was carried out using the WinQxas software (International Atomic Energy Agency, 1997-2002).

Copper (Cu), zinc (Zn), iron (Fe) and molybdenum (Mo) were the most abundant metals detected in all samples. Concentrations (on a wet wt. basis) ranged between 4 and 503 mg/kg for Cu (mean value 66±85 mg/kg), between 18 and 350 mg/kg for Zn (mean value 86±57 mg/kg), between 16 and 1348 mg/kg for Fe (mean value 151±184 mg/kg) and between 0.27 and 5.15 mg/ kg for Mo (mean value 1.51±1.05 mg/kg). These results compare well with literature data reported worldwide.

Elemental concentrations were log-normally distributed and thus the data were log-transformed for further statistical analysis. Significant correlations ($p<0.05$) were found only between Fe and Zn ($r=0.37$) and between Cu and Zn concentrations ($r=0.28$). No correlation was found between Cu and Mo concentrations. However, molybdenum is a metabolic antagonist to copper and interferes with the Cu metabolism by restricting the absorption of Cu from the gut and its storage in the liver and kidney. It is well established that high levels of Mo induce Cu deficiency, while low levels of Mo induce Cu poisoning. The copper status of sheep is therefore more related to the Cu:Mo ratio than to the levels of the single elements. Indeed, a significant positive correlation was established from the present data between Cu:Mo ratio and Cu levels ($r=0.83$, $p<0.001$).

One-way analysis of variance (ANOVA), carried out to determine any significant differences between animal species, showed that Cu concentrations were significantly higher ($p<0.001$) in sheep (79±90 mg/kg) compared to goats liver (9.4±4.1 mg/kg). Significant difference ($p<0.001$) between sexes was established only for Mo levels (1.67±1.05 mg/kg for female, compared to 0.62±0.39 mg/kg for male animals). The animal age (either <1.5 y or >1.5 y) had a statistically significant ($p<0.001$) effect only in Mo levels (0.76±0.52 mg/kg in younger animals, compared to 1.96±1.02 mg/kg in older ones).

Keywords: *Heavy metals, liver, sheep, goat, Greece, EDXRF spectrometry*

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F-40
SINGLE DROP MICROEXTRACTION OF DERIVATIZED PARABENS

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Parabens are the esters of p-hydroxybenzoic acid. Due to their antibacterial and anti-fungal properties they are widely used as preservatives in cosmetics, personal care and pharmaceutical products, so they are continuously released in to the environment [1]. Parabens have a negative effect on human health [2], thus there is an increasing interest in their trace analysis. Gas chromatography is one of the most common methods for parabens analysis. However, in order to determine low concentrations of parabens, prior to the analysis it is necessary to apply a preconcentration step. Single drop microextraction (SDME) is a miniaturised version of liquid phase extraction. In the simplified version of this method, the microdrop of an appropriate solvent is suspended by microsyringe in the stirred aqueous sample solution [3] or held in the headspace above the solution [4]. The extraction drop remains on the tip of the syringe for a set extraction time after which the drop is withdrawn into the syringe and injected into a gas chromatographic system. Due to their polar nature, prior to gas chromatographic analysis parabens are often derivatized to reduce their adsorption in the chromatographic system, to improve sensitivity, peak separation and peak symmetry. A convenient derivatization approach is in situ acylation by acetic anhydride [5]. In the present work, a simple and rapid single drop microextraction method with in situ derivatization and gas chromatographic determination of parabens was develop. A dependence of the derivatization efficiency on pH was investigated and it was determined that the optimum pH is 9. Six potential extraction solvents were investigated and it was determined that amyl acetate is the best extraction solvent for direct SDME and dioctylphthalate is the best extraction solvent for headspace SDME. For the both SDME modes extraction time, extraction temperature and salt concentration in the solution were optimized. At optimum extraction conditions the precision, linearity and detection limits were determined. A possibility to apply the proposed methods for parabens determination in real samples was demonstrated.

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Keywords: *Parabens, food contaminants, single drop, microextraction, gas chromatography*

F-41
EFFECTS OF "MASTER CURVE CALIBRATION" ON THE PERFORMANCES OF SOME ELISAS FOR FOOD CONTAMINANTS

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Thanks to their reliability, sensitivity and precision, ELISAs are widespread for screening a number of contaminants in different food and feedstuffs. The analysis is based on the reaction between a binder (typically, an antibody) and one or more ligands. Such binding can be affected by environmental conditions (i.e. the temperature) as well as degradation of binders and/or tracers; the analyst is therefore generally required to run a calibration curve within each analytical session, even when analysing one or a few samples. The aim of the present work was to develop a new generation of quantitative microplate immunoassays for chemical contaminants with no need to run any calibrator apart the "zero" standard. Proper calibration curves, which B/Bo values are optimised for each batch, have been established ("master curves"). Once obtained the ratio between each sample absorbance (B) and the "zero" standard absorbance (Bo), the samples' B/Bo values have been interpolated onto the master curve to obtain the quantification of the analyte. By testing the same sample extracts in parallel, the verification of the performance was carried out by comparison with that of the traditional kit. For the present study, two fast ELISA test kits for the quantitative detection of deoxynivalenol and aflatoxin B1 were selected: Celer DON v2 and Celer Afla B1, respectively. The "master curve" versions were named B ZERO DON and B ZERO Afla B1. The sample preparations were the same. Blank and spiked maize and wheat samples were run in parallel onto Celer and B ZERO. No significant differences were obtained in terms of specificity and sensitivity, apart from a slight loss of sensitivity when testing maize by B ZERO Afla B1. The accuracy was assessed by testing control materials: both Celer and B ZERO formats led to satisfactory results. Intra- and inter-assay precision did not show significant worsening when B ZERO analysis was performed. The "master curve" format was applied also to a receptor assay for tetracyclines: SuperScreen Tetra HS, a quantitative broad-range assay for the detection of tetracycline, chlortetracycline, doxycycline and oxytetracycline. The scope was to verify whether the use of the master curve could affect the performance of a binding assay where a receptor is used instead of an antibody. Moreover, the B ZERO Tetra HS has to be run in single well, while the original test kit asks for duplicate wells. Again, no significant differences in terms of specificity, sensitivity and mean concentration values were obtained. As a conclusion, thanks to the stability of the reagents and to the assays robustness, the B ZERO kits performed as the relative original kits. The use of the manufacturer master curve makes the B ZERO kits the most suitable option for those testing a few samples per run: the number of wells available for sample analysis on the microtiter plate is higher, making the cost of each test much lower.

Keywords: *ELISA, calibration, mycotoxins, tetracyclines, ruggedness*

F-42
RESULTS FOR PAH ANALYSIS ARE NOT METHOD-DEPENDENT IN PROFICIENCY TESTS

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An assessment of laboratory results from two independent proficiency test providers for PAH analysis was conducted to assess for possible method dependency. The results of the investigation could give laboratories confidence in their current approach and offer reassurance that no alternative instrumentation is required in their existing facilities. Proficiency test results from FAPAS, and the other under the auspices of the European Union Reference Laboratory for PAHs analysed datasets for the four EU marker PAHs; benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene. Laboratory results were separated based on whether gas chromatography or liquid chromatography had been used. The investigation showed that there was no significant difference in results between laboratories using the different approaches. The absence of general good laboratory practice or asymmetrical distribution was attributed as the cause for occasional differences. The se results offer assurance that laboratories using either approach are delivering the quality results demanded by their customers.

Keywords: *FAPAS, Proficiency Testing, PAH*

F-43
DESIGN OF EXPERIMENT APPROACH (DOE) FOR THE OPTIMIZATION OF POLYBROMINATED DIPHENYL ETHERS (PBDEs) DETERMINATION IN FISH SAMPLES BY MICROWAVE-ASSISTED EXTRACTION AND GC–MS/MS

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A design of experiment approach (DoE) [1] was used for the optimization of large volume injection (LVI) [2] and microwave-assisted extraction (MAE) [3] parameters for the analysis of PBDEs (BDE-28, BDE-49, BDE-47, BDE-66, BDE-100, BDE-119, BDE-99, BDE-155, BDE-154, BDE-153, BDE-139 and BDE-183) in fish samples using GC-MS/MS. A central composite design (CCD) was used to build the response surface for each PBDE compound, and find the factor settings that maximize the injection and extraction efficiency for all analytes using the MINITAB software. For the optimization of the LV injection, the most important parameters were: injection temperature (Tinlet), vaporization temperature (Tvap), vaporization time (tvap) and evaporation flow (Flow). Results showed that the optimal conditions were Tinlet = 50°C, Tvap = 80°C, tvap = 2 min, and flow = 10 mL/min. For the optimization of MAE parameters, the three main factors were solvent volume, exposure time and temp erature and their optimal values were: 50 mL, 2 min., 75°C, respectively. In addition to both optimizations by DoE approach, other conditions of the method were studied: GC–MS/MS parameters, extraction solvent and matrix effect. The final method consisted on the extraction using MAE with n-hexane:acetone (1:1, *v/v*), followed by acid treatment and SPE clean-up, and GC–MS/MS determination. Separation was carried out on a HP-5MS capillary column, 30 m × 0.25 mm i.d., 0.25 µm film. The oven temperature program was as follows: initial 80°C (2 min); 25°C min^{-1} to 230°C; 5 °C min^{-1} to 320°C (5 min). The mass spectrometer operated in the electron impact mode (EI) using a voltage of 70 eV and a filament current of 50 µA. The ion source temperature and the transfer line temperature were set at 250°C and 300°C, respectively. Validation of the method was achieved by the study of linearity, accuracy, precision and limit of quantification (LOQ). Linearity was accomplished with matrix-matched calibration and internal standards (BDE-28 $^{13}C_{12}$, BDE-47 $^{13}C_{12}$, BDE-99 $^{13}C_{12}$ and BDE-153 $^{13}C_{12}$), in the range of 0.5–100 ng/mL in vial. Accuracy and precision were studied in terms of recovery and relative standard deviation (RSD) by analyzing spiked blank samples at two different concentrations: the LOQ level (0.05 µg Kg^{-1}– 0.94 µg Kg^{-1}) and the higher level of the calibration curve (9.40 µg Kg^{-1}). Mean relative recoveries at LOQ level ranged from 77% (BDE-183) to 111% (BDE-99) with relative standard deviation below 20%. The validated method was tested with twelve real samples.

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Keywords: *PBDEs, Experimental Design, Programmed Temperature Vaporizer (PTV), Microwave Assisted Extraction (MAE), GC-MS/MS*

F-44
DETERMINATION OF DICYANDIAMIDE BY LC–MS/MS IN DAIRY INGREDIENTS: A RESPONSE TO A DECISION OF THE MINISTRY OF PRIMARY INDUSTRIES IN NEW ZEALAND

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In January 2013, the Ministry of Primary Industries (MPI) in New Zealand issued a press release in support of New Zealand companies withdrawing fertilizer containing dicyandiamide (CAS 461-58-5, DCD) from distribution in New Zealand. DCD was used since 2004 for direct application to pasture as the more promising way of reducing nitrate leaching to waterways and greenhouse gas emissions from farming, as well as promoting pasture growth. DCD does not present a food safety issue, as evidenced by results of studies of the United Nations Environment Programmes (NOAEL at 1 g/kg b.w./day in rat) and the European Scientific Committee for Food (TDI at 1 mg/kg b.w./day). However, the findings of DCD in milk products were unexpected and the MPI decided the withdrawal of the fertilizer while more research is undertaken. As a response to this situation, a liquid chromatography-tandem mass spectrometry (LC–MS/MS) method was developed shortly at Nestlé Research Centre in order to provide Markets with a tool to assess the presence of DCD in dairy ingredients. The analytical procedure encompasses a reconstitution of dairy powder in water, followed by a dilution with acetonitrile and subsequent acidification and a partitioning with sodium chloride upon centrifugation. A portion of the resulting supernatant is washed with hexane and finally analyzed by LC-MS/MS in SRM using the positive electrospray ionisation mode. A hydrophilic-lipophilic (HILIC) chromatography column is used for analyte chromatography. Quantification is performed by the isotopic dilution approach using $^{15}N_4$-DCD as internal standard. A survey of > 220 milk-based ingredients from New Zealand revealed that 77% of the samples were below the reporting limit at 0.05 mg/kg, 22% of the results in the range 0.05 – 1.0 mg/kg, and the highest value recorded at 1.39 mg/kg. The amounts of DCD found in food ingredients are not a food safety issue. However, like melamine, DCD has recently been added to a list of compounds that might be intentionally used as an economic adulterant to artificially elevate the apparent protein levels in milk; for this reason, health authorities such as the US FDA have developed analytical methods to detect DCD in foodstuffs. Today, there is no reported evidence that DCD has been employed as a food adulterant.

Keywords: *Farming, dicyandiamide, LC-MS/MS, dairy ingredients*

F-45
IMPROVED APPROACH FOR THE DETERMINATION OF PERSISTENT ORGANIC POLLUTANTS (POPs) IN FATTY FOODS AND BEVERAGES USING QUECHERS EXTRACTION/CLEANUP AND GC/MS

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Persistent organic pollutants (POPs) are harmful man-made compounds that are not easily degraded by chemical, biological, or photolytic processes. Therefore, they persist in the environment for long periods of time. POPs encompass compound classes such as dioxins, polychlorinated biphenyls (PCBs), chlorinated pesticides, and polyaromatic hydrocarbons (PAHs). Many of these compounds are lipophilic, and bioaccumulate in the fatty tissues of living organisms. Moving up the food chain, their concentration increases as they pass from one organism to another [1]. As a result, these compounds can end up in animal-based foods such as fish, meat, and milk. The analysis of POPs in fatty foods is challenging, because fats often get co-extracted with the target analytes, causing interference and/or sensitivity issues during analysis. Work done recently by Sapozhnikova and Lehotay on extraction and analysis of POPs in catfish, found zirconia-coated silica effective in ba ckground removal, while obtaining good recovery and reproducibility [2]. An additional source for PAHs in food may be the grilling process, because PAHs can be formed during the grilling process when the flames containing the PAHs come into contact with the meat and the PAHs get absorbed into the food. The amount of PAHs in grilled food products varies greatly with the type of meat, the cooking temperature, and how long the meat is cooked [3]. Extracting PAHs from complex matrices like animal meat that contain fats, lipids, muscles, and proteins can involve multi-step extraction procedures that are time-consuming and complicated. In this work, the QuEChERS method (dispersive SPE), developed by Anastassiades and Lehotay [4], using two new zirconia-coated silica adsorbents was applied for the extraction and cleanup of PAHs from raw salmon and grilled burger as well as for PCBs from cow's milk. For GC analysis of these compounds capillary columns were especially selected to provide the optimum selectivity, temperature range and analysis time. The use of the zirconia-coated silica resulted in higher removal of matrix components by an improved recovery for the POPs.

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Keywords: *POPs, PCBs, PAHs, QuEChERS, Contaminants*

F-46
OPTIMIZATION OF QUECHERS EXTRACTION METHOD FOR THE DETERMINATION OF EDCS IN DAIRY PRODUCTS VIA LC–HYBRID LTQ ORBITRAP MS

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Food quality and safety is of paramount importance to global economy and individual health. In present study a simple method for the determination of some Endocrine Disrupting Chemicals (EDCs) including alkylphenols and bisphenol A residues in fatty food was developed, taking advantage of the innovative hybrid technology and versatility of the LTQ FT Orbitrap MS. Target analytes were selected in terms of the frequency of their presence in the environment and their known appearance during processes and procedures leading to animal origin food products. The presence of their residues in food commodities can irreversibly affect the consumer's health. Therefore, an urgent demand appears for the rapid and robust detection of these EDCs in even such complex matrices as fatty food so that regulatory limits may be enforced. U–HPLC–LTQ FT Orbitrap MS was employed for that purpose demonstrating excellent sensitivity and enabled the high mass resolution and accuracy identification of all compounds. Detection of 4 industrial chemicals (negative ion mode) at low ppb levels (LOQ< 2ppb) was achieved within 10 min. All target analytes exhibited excellent linearity. In all cases precision was lower than 6%, expressed as RSD. QuEChERS has been evaluated for the extraction of these compounds in dairy products. This method involves microscale extraction using a small volume of acetonitrile and dispersive solid-phase extraction (d-SPE) with a major reagent and relies on the difference in affinities between the reagents and analytes. Furthermore different sorbents have different affinities for the analytes. Experimental design approach was employed for screening as well as effect estimation of different parameters, such as the extraction solvent, the adsorbent and the sample size on the method extraction yield, in order to find the optimum conditions for the method application to real samples. In general, the sensitivity obtained meets the maximum residue levels (MRLs) established by the European Union regulation for food monitoring programs.

Keywords: *EDCs, dairy products, QuEChERS, Orbitrap*

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F-47
ANALYSIS OF QUATERNARY AMMONIUM COMPOUNDS IN VARIOUS DAIRY PRODUCTS BY LC–MS/MS

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Quaternary ammonium compounds (QACs) carrying long alkyl chains as substituents possess antimicrobial activity and are thus frequently used in disinfectants. Benzalkonium and dialkyldimethylammonium chlorides are QACs that are widely used for disinfection purposes in the food industry. Upon their application residues of these substances may be found in various foodstuffs, especially in milk and milk products, as there is a strong need for disinfection throughout the dairy production chain. We have developed a method for the analysis of nine QACs in dairy products employing rapid sample preparation, followed by measurement with LC–MS/MS. The impact of the QACs' specific chemical properties (permanent positive charge and extended hydrophobic moiety) on the chromatographic separation and mass spectrometric detection will be discussed and insights gained from optimisation of both LC and MS/MS will be touched upon. The final method which provided rapid sample throughput was validated and yielded highly satisfactory characteristics in terms of trueness (determined as recovery) and precision (determined as intermediate precision). Moreover, it is highly sensitive with limits of quantification in the sub-ppb range for all QAC analytes. The method was applied to the analysis of a wide range of various dairy products (milk, milk powder, ice-cream) from the Austrian market. The results obtained for the various QACs will be presented and discussed with respect to legislative limits and regarding the potential entry of these compounds into dairy products at different stages of the production chain.

Keywords: *Quaternary ammonium compounds, LC–MS/MS, dairy products*

F-48
DETERMINATION OF POLYETHOXYLATED TALLOW AMINE (POEA) WITH HIGH RESOLUTION AND ACCURATE MASS SPECTROMETRY IN FOOD AND FEED

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Surfactants, such as polyethoxylated tallow amine (POEA) in herbicide formulation are intended to improve the wettability of the hydrophobic surface of plants for maximum coverage and to aid penetration through the plant surface. In some herbicide products, a surfactant is also needed as a solubilizer. POEA does not have clearly defined chemical composition. It is produced of fat from the fatty tissue of cattle or sheep (tallow). This is the reason why POEA contains a variety of fatty acids, like oleic (37–43%), palmitic (24–32%), stearic (20–25%), myristic (3–6%), linoleic (2–3%) acids and other minor compounds in different amounts. The difference in the raw material is responsible for the varying number of carbons in the hydrophobic tail of the structurally related compounds. The surfactant POEA is mostly used in glyphosate formulations. The variability in the chemical nature of the surfactant in the formulated end-use product makes it difficult to ascertain which is more toxic, the surfactant or the herbicide glyphosate itself. A review of the literature provided to the EPA in 1997 found that POEA was generally more potent in causing toxicity than glyphosate. The chemical complexity of POEA makes it difficult to study them in feed and food. In the recent years, several studies tried to analyse POEA with low resolution LC–MS or LC–MS/MS. It is reported that only a semi-quantitative approach is possible due to their different fatty acid composition. Another difficulty of the low resolution LC–MS is the limited mass accuracy and resolution, or with the LC–MS/MS the limited number of mass traces and pure targeted nature of selected reaction monitoring technique. With high resolution and an accurate mass LC–MS instrument like the Orbitrap based Exactive PlusTM, it is possible to identify the different tallow compositions and to propose an elemental composition of the detected compounds. As a result, a more reliable esti mation of the POEA concentration is feasible due to the possible summation of all identified constituents rather than the consideration of only a limited number of mass transitions.The semi- quantitative analysis of POEA down to a concentration of 50 µg/kg in a product like corn or soya bean is possible. Consequently, a POEA analysis in food and feed in a comparable concentration range as of glyphosate residues in such products is feasible. With high resolution and accurate mass spectrometry using an Exactive PlusTM, it is possible to unequivocally monitor these compounds easily. Additional benefits are very simple instrumental setup, as the HRMS instruments operate in full scan mode and compound specific setup is avoided.

Keywords: *Polyethoxylated tallow amine (POEA), high resolution, herbicide formulation, surfactant*

F-49
MITIGATION OF STYRENE IN WHEAT BEER

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2-Methoxy-4-vinylphenol and 4-vinylphenol are important aroma compounds in wheat beer, which are formed from their respective phenolic acids (ferulic and p-coumaric acid) after thermal or enzymatic decarboxylation during the brewing process. But, by this way, also the "food-borne toxicant" styrene, classified as "possibly carcinogenic to humans" (group 2 B) by the International Agency for Research on Cancer (IARC), can be generated from cinnamic acid. Therefore, it is a fundamental task for breweries to produce wheat beers with reduced styrene concentrations without changing the aroma. Thus, the aims of this study were (i) to analyze the concentrations of free phenolic acids (precursors) in wheat and barley malts produced with different malting parameters (steeping degrees, germination times, and germination temperatures) and (ii) to analyze the concentrations of the undesirable styrene and the desirable vinyl analogous aroma compounds (2-methoxy-4-vinylphenol and 4-vinylphenol) in beers produced from these malts. Quantitation via Stable Isotope Dilution Analysis (SIDA) showed that, in general, higher malting parameters led to higher concentrations of free phenolic acids in the malts. For example, a barley malt produced with the malting parameters 42% steeping degree, germination temperature 12°C, and germination time 5 days showed lower concentrations of precursors (0.73 mg of p-coumaric acid/kg dry mass, 2.62 mg of ferulic acid/kg dry mass, and 0.67 mg of cinnamic acid/kg dry mass) in comparison to a barley malt produced with the malting parameters 48%, 18°C, and 8 days (1.47 mg of p-coumaric acid/kg dry mass, 4.09 mg of ferulic acid/kg dry mass, and 1.54 mg cinnamic acid/kg dry mass). By using malts with lower malting parameters and, thus, somewhat lower concentrations of free precursors for brewing, the styrene concentration in a model wheat beer can be reduced. For example, a green beer produced from 100% wheat malt with the malting parameters 42%, 12°C, and 5 days only contained 17.5 µg/L styrene, whereas a green beer produced from wheat malt with the malting parameters 48%, 18°C, and 8 days contained 25.4 µg/L styrene.

Keywords: *Styrene, wheat beer, malt*

F-50
THE PHARMACOKINETICS OF TETRACYCLINE IN *BRASSICA CHINENSIS* L. GROWN HYDROPONICALLY

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Veterinary antibiotics are increasingly being monitored in slurry, soils and surface waters and have been reported to contain antibiotic concentrations ranging from several to hundreds of milligrams per liter. The most commonly detected antibiotics in soil and river in Taiwan are sulfonamides, tetracyclines and lincosamides, the concentrations range from 1.6 to 112 µg/L. Antibiotics uptake by plants in soil and water matrix becomes not only an environmental but also a human health concern. The pharmacokinetics (uptake, distribution, metabolism and elimination) of tetracycline (TC) in edible vegetable *Brassica Chinensis* L. was investigated in this study. The vegetable was exposed to 100 µg/mL TC in cultivation water for up to 24 hrs, drug concentrations in roots, stems and leaves were quantified using optimized HPLC–UV method and the bioaccumulation factors (BAF) in various parts of the vegetable were calculated. The results showed that TC concentration in the root was as high as 1200 µg/mL, indicating preferential accumulation of the drug in the root (BAF=21 at 24 hr). TC concentration in the leaves was around 55 µg/mL while only 15 µg/mL was detected in the stem (BAF

Keywords: *Tetracycline, pharmacokinetics, bioaccumulation factor, Brassica Chinensis L*

F-51
DETERMINATION OF HEAVY METAL CONTENTS IN INDIAN MARINE FISH USING ICP–MS METHOD AFTER CLOSED VESSEL MICROWAVE DIGESTION

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The marine foods including fish and shellfish are one of the major sources of animal proteins, nutrients and are particularly valued for their omega 3 fatty acids, which is reported to reduce the risk of cardio-vascular diseases, stroke and preterm delivery. India with its vast coastal line has tremendous potential in terms of marine food resources with several species available for human consumption. Heavy metals such as Nickel (Ni), Arsenic (As), Cadmium (Cd) and Lead (Pb), exerts toxic biochemical and functional effects even at low levels and consequently affects hematopoietic system, central nerve system (CNS), liver nd kidneys in humans. Data pertaining heavy metal content of edible fish and shell fish of Indian market are scarce. Therefore a study was undertaken to precisely evaluate the concentration of such toxic HMs in large variety of fish and shell fish collected from the outlets of twin cities of Hyderabad and Secunderabad, Andhra Pradesh, India, using Inductively Coupled Plasma Mass Spectrophotometer (MD–ICP–MS) after closed vessel microwave digestion. Compared to Colorimetric, AAS and ICPAES methods that are available for metal analysis, ICP–MS is the most sophisticated, reliable, fast, sensitive, selective, precise, accurate quantitative technique for monitoring heavy metals in food samples with feasibility of isotope ratio measurements. HM content ranged from 61 to 153 µg/kg of Nickel in Lates calcarifer and Mugil cephalous; 28 to 4770µg/kg of Arsenic in Rama chandramara and Scoliodon sorrakowah; 3 to13 µg/kg of Cadmium in Lates calcarifer and Scomberomorus guttatus and 17 to 703 µg/kg of Lead in Katsuwonus pelamis and Mugil curema marine fishes respectively. These HMs like Ni, Cd and Pb are within the permissible, tolerable limits as suggested by WHO/FAO (eccept Arsenic). Thus this new data generated on HMs is useful for researchers and policy makers.

Keywords: *Marine fish, Heavy metals,ICPMS*

F-52
ANALYSIS OF BENZO(A)PYRENE AND PAH IN VEGETABLE OILS

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Lipid complex of vegetable oils is an accumulator of xenobiotics, in particular - polycyclic aromatic hydrocarbons (PAHs). Therefore, to ensure safe use of vegetable oil and food based on it requires a constant analytical control of PAHs. The aim of this work was to ensure control of the content of benzo(a)pyrene and polycyclic aromatic hydrocarbons in vegetable oils according to modern sanitary requirements. The objects of research are vegetable oils and hydrogenated vegetable fat. Analysis of PAHs is performed by donor-acceptor complex chromatography HPLC with fluorescence detection. The analysis time per sample is approximately 70 min. Compared with other methods, this method of PAHs control is characterized by high sensitivity, requires no sample preparation of the sample, which allows to eliminate the loss of analyte. In this method of analyzing were applied the sunflower, corn, olive, flaxseed, pumpkin seed oil, were determined concentration of PAHs eliminating the step of alkaline hydrolysis of the sample. Range of concentrations of PAHs was 0.5 mkg/kg and 10.0 mkg/kg. Technique meets the requirements of DSTU 17025 and EU SANCO 12495-2011, which is confirmed by the appropriate validation characteristics. The developed method has been successfully applied in the meet the following inter-laboratory comparisons, performing routine analyzes for 2011–2013. According to the results of measurements crude vegetable oils are always contained four PAHs: benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene. The mass ratio of these contaminants are usually different.

Keywords: *PAH, vegetable oils, HPLC*

F-53
OCCURRENCE OF ORGANOHALOGENATED COMPOUNDS AND PAHs IN FISH FROM TURKEY PART A: UHPLC–MS/MS DETERMINATION OF PFASs, BFRs AND THEIR HYDROXYLATED METABOLITES IN FISH

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The aquatic ecosystem is due to human activity exposed to a large number of various chemicals which results in negative changes of the water quality, threating aquatic organisms and via food chain also human health. Perfluoralkyl substances (PFASs) and brominated flame retardants (BFRs) belong to one group of these hazardous compounds which contaminate the environment and thus could negatively affect the living organism including humans. The presented study was focused on the monitoring of pollution of selected areas in Turkey (Marmara Sea, Aegean Sea and Black Sea) by PFASs and BFRs, namely hexabromocyclododecanes (HBCDs), tetrabromobisphenol A (TBBPA), brominated phenols and hydroxylated polybrominated diphenylethers (OH-PBDEs). Altogether 15 samples of fish muscle tissue, represented by five different fish species were examined. The used sample preparation procedure based on extraction using acetonitrile and subsequent purification by dispersive solid-phase extraction (d-SPE) with C18 sorbent is rapid, simple and high-throughput. Using modern instrumentation consisting of ultra-high performance liquid chromatography (UHPLC) interfaced with a tandem mass spectrometry (MS/MS), limits of quantification (LOQs) in the range of 0.01–0.02 ng/g for PFASs and 0.01–0.60 ng/g for BFRs were achieved [1, 2]. From 18 target PFASs, only 10 analytes exceeded LOQ in tested samples. The total concentration of PFASs in fish ranged from 0.33 to 3.15 ng/g (median 1.18 ng/g). The most abundant representatives were perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (PFOSA), which were found in more than 60% of samples. In the case of BFRs, only 2 brominated phenols were detected in the range of 0.17–3.21 ng/g. The highest concentration of organohalogenated compounds were found in fish originated from the Marmara Sea and Black Sea. No contamination was detected in samples from the Aegean Sea.

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Keywords: *BFRs, PFASs, OH-PBDEs, fish, UHPLC–MS/MS*

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F-54
OCCURRENCE OF ORGANOHALOGENATED COMPOUNDS AND PAHs IN FISH FROM TURKEY PART B: GC–MS/MS DETERMINATION OF HALOGENATED POPs AND PAHs IN FISH

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Within this study, the set of five fish species (Horse mackerel, Red fish, Red mullet, Gilthead seabream and Sea bass) originated from three areas in Turkey (Marmara Sea, Aegean Sea, Black Sea) was investigated for the presence of POPs, represented by polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and polybrominated diphenylethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs). The sample preparation procedure was based on an ethyl acetate extraction of wetted sample followed by silica minicolumn clean-up. The gas chromatography (GC) interfaced with tandem mass spectrometry (MS/MS) was employed for the identification/quantification of target compounds [1, 2]. In summary, in all examined samples at least 18 target analytes (from the total of 57 compounds involved within the analytical method) were detected; however, no sample exceeded the limits set in the EU legislation for sum of dioxin-like PCBs and sum of major six non-dioxin like PCBs (CB 28, 52, 101, 138, 153 and 180) in fresh fish meat. Regarding to the total concentration of individual analytes groups, the mean concentrations decreased in the following order: ΣOCPs (33.3 ng/g)>ΣPCBs (9.86 ng/g)>>ΣPBDEs (0.28 ng/g)~ΣPAHs (0.12 ng/g). CB 153, CB 180 and p,p'-DDE were the most frequently found contaminants at the concentrations: 0.20–8.61, 0.09–2.79 and 0.38–27.6 ng/g, respectively. The highest amount of POPs was quantified in Sea bass originated from the Black Sea. The different levels of contamination within the same area, namely Marmara Sea, and various species of fish were found. The total concentration of POPs decreased in the following order: Sea bass>Red mul let>>Gilthead seabream~Horse mackerel>Red fish. Not only these POPs were examined, but also liquid chromatography (LC) amenable contaminants, namely perfluoralkyl substances (PFASs), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA) and several hydroxylated metabolites of BFRs were involved within our study (for detail, please see PART A: UHPLC–MS/MS DETERMINATION OF PFASs, BFRs AND THEIR HYDROXYLATED METABOLITES IN FISH). In regards to the amount of ΣPFASs within different fish species, the highest concentration (2.7 ng/g) was found in Horse mackerel followed by Red fish~Sea bass~Red mullet>>Gilthead seabream (opossed to previously mentioned POPs).

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Keywords: *POPs, PAHs, GC–MS/MS, fish*

Acknowledgement: *The financial support from the specific university research (MSMT No 20/2013) is acknowledged. Thanks to HPST for the delivery of fish samples.*

FOODOMICS
(G-1 – G-8)

G-1
BACK-TRACING AN EMERGING ENVIRONMENTAL TOXICANT (HEXABROMOCYCLODODECANE, HBCD) IN ANIMAL-DERIVED FOOD CHAIN BASED ON FOODOMICS

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Human activity is the main cause of the emission of pollutants which may accumulate in environmental compartments, then in livestock tissues and subsequently in the food chain. The toxic environmental micropollutants possibly transferred to animal products are listed in the frame of the Stockholm Convention. These chemicals are particularly monitored in food products and include brominated flame retardants. Among brominated flame retardants, hexabromocyclododecane (HBCD) is a chemical of potential concern currently proposed for listing.
HBCD is an emerging toxic micropollutant found in the environment and in animal tissues. Direct HBCD quantification is extremely difficult because it undergoes a rapid metabolism in biota. Based on a previous report showing the relevance of volatile compound metabolic signature in chicken liver for back-tracing a dietary exposure to rapidly metabolized xenobiotics [1], the present study investigates the relevance of this approach to evidence a previous HBCD contamination in laying hens.
Three groups of laying hens (n=56) were fed a similar feed either non-contaminated (control group) or contaminated during 71 days with either 0.1 $\mu g.g^{-1}$ or 10 $\mu g.g^{-1}$ HBCD. Animals were periodically slaughtered throughout the experiment and their liver was excised. Solid phase microextraction – gas chromatography-mass spectrometry (SPME–GC–MS) was used to determine the liver content in volatile compounds.
After correcting the data from instrumental drifts by normalization methods, the use of volatile compounds enabled the differentiation of samples according to contamination level of animals. For a same contamination level, a discrimination of the samples according to exposure duration was also observed. This discrimination is improved when animals are exposed to the highest HBCD dose.
The volatile compound metabolic signatures in poultry liver seem to be relevant in order to back-trace an exposure to HBCD in laying hens. In order to validate the metabolomic approach and to enable its use in the field conditions, further investigations are undertaken to determine the biochemical pathways responsible for the changes in the levels of these volatile biomarkers.

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Keywords: *Hexabromocyclododecane, food safety, laying hens, SPME-GC-MS, data normalization*

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G-2
TOWARD NEW COMPREHENSION OF CHEESE RIPENING

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Metabolic fingerprinting is an untargeted approach used in many scientific areas, which has shown potential to investigate food quality and safety [1]. So far, this approach has not been applied to cheese. This study aimed to investigate the ability of mass spectrometry (MS) metabolic fingerprinting to characterize the modifications induced by bacterial metabolism in cheese over time [2]. Metabolic fingerprints were acquired after 0, 8 and 48 hours of incubation from two different fractions of the cheese metabolome: i) a water-soluble fraction, analyzed using liquid chromatography-high resolution-MS and ii) a volatile fraction analyzed using gas chromatography-MS. MS metabolic fingerprints were found to differ significantly depending on the incubation time, pointing out the capacity of this approach to study the evolution of bacterial metabolism within cheese. Forty-five metabolites were identified on the basis of an internal data bank [3]. Variations over time of a large diversity of well-known cheese metabolites such as 12 amino acids and 25 volatile compounds, but also less studied ones such as 4 vitamins and L-carnitine were highlighted. These results showed the relevance of cheese MS fingerprintings to detect even slight differences and to possibly generate new insights on little-known cheese metabolites. As perspectives, these complementary untargeted "omic" approaches could be applied to explore strain biodiversity and the influence of ripening factors on microbial metabolism.

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Keywords: *Metabolomics, Cheese, Ripening, Biomarkers*

G-3
AUTOMATIC IDENTIFICATION OF UNKNOWN AND UNEXPECTED CHEMICAL RESIDUES AND CONTAMINANTS IN FOOD SAMPLES USING ACCURATE MASS LC–MS/MS SCREENING TECHNIQUES

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Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC–MS/MS using an AB SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode. Full scan MS and MS/MS data was explored to identify known-unknowns using non-targeted data processing tools. Sample-control-comparison was successfully used to find unexpected contaminants. Identification was based on MS and MS/MS information, including formula finding, ChemSpider searching, and automatic MS/MS fragment ion interpretation. This challenging data processing workflow was automated and allows easy result review and reporting in the latest revision of TripleTOF® software.

Keywords: *Unknown screening, identification, software, Time-of-flight, LC–MS/MS*

G-4
SYSTEMATIC DETERMINATION OF BIOMARKERS BY AUTOMATED PROCESSING OF GAS CHROMATOGRAPHY–MASS SPECTROMETRY DATA

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Gas chromatography-mass spectrometry is now widely used in metabolomics but the comprehensive determination of potential biomarkers remains challenging. The development of tools for an automatic search of markers requires breaking down several barriers including noise filtering, signal warping and normalization. The present paper introduces a set of tools for an automatic processing of chromatography mass spectrometry data and a comprehensive determination of distinctive biomarkers. This toolkit includes a novel warping technique enabling correction of local distortions. Beyond alignment issues, the approach implements several chemometric methods which were recently developed for noise correction, data normalization [1,2] as well as an original tool designed for peak deconvolution and marker identification [3]. The relevance of our approach and its performance were assessed on a set of GC–MS signals (n=117) corresponding to the volatile compounds analyses o f three vegetable oils after dynamic headspace extraction [1]. The robustness of the biomarkers determined by our automated approach was successfully evaluated by a comparative test with a marker list produced by a mass spectrometry expert.

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Keywords: *Gas chromatography–mass spectrometry, biomarkers, chemometrics, signal processing, warping*

G-5
USE OF BIOANALYTICAL STRATEGIES FOR ZINC FRACTIONATION IN MUSCLE TISSUE SAMPLES OF NILE TILAPIA FED WITH ORGANIC AND INORGANIC ZINC SOURCES

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In last years, new studies with proteomic in fish have been developed to assist animal nutrition according to increase in production of these animals [1]. In nutrition, minerals are important in animal metabolism because many of them are cofactors of proteins as in the case of zinc with some proteins as lactate desidrogenase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase, superoxide dismutase, in the case of zinc. In the metalloproteomic studies, the two-dimensional electrophoresis appears as an important tool in proteins separation with great definition and then the mineral is identify by graphite furnace atomic absorption spectrometry (GFAAS) [2]. Considering the above, in the present study, the polyacrylamide gel electrophoresis 2D-PAGE was used for obtain the proteome of muscle tissue samples of Nile Tilapia fed with organic and inorganic zinc sources in diet in two phases of development (24 g and 85 g) and further been done the mapping zinc in protein spots obtained from the proteins fractionation by atomic absorption spectrometry in graphite furnace. The zinc concentration ranged from 0.47 to 55.91 ppb for animals of 24 g fed with organic zinc; 51.78 to 118.54 ppb for those of 24 g fed with inorganic zinc; 84.69 to 228.33 ppb and 1.64 to 30.09 ppb for the animals of 85 g fed with organic and inorganic group respectively. And as for the isoeletric point (pI) and molar mass (MM) to fishes of 24g were from 3.0 to 7.30 and 12.09 to 57.57 kDa for those fed with organic zinc; 3.01 to 6.41 and 22.24 to 95.49 kDa for those fed with inorganic zinc. And to fishes of 85 g were from 3.16 to 7.03; 10.09 to 57.06 kDa for organic and 3.06 to 6.36; 20.98 to 94.31 kDa for inorganic. Thus it is observed that the sources of zinc can influence in metalloproteome of muscle tissue of fishes, whereas zinc was identified in different spots and consequently, with different isoeletric points and molar mass, changing their concentrations acco rding to the source of the mineral present in diet.

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Keywords: *Sources of zinc, metalloproteomic, 2D-PAGE, GFAAS*

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G-6
INFLUENCE OF CULTIVATION CONDITIONS ON THE GROWTH OF CULTURES AND PRODUCTION OF ORGANIC ACIDS IN ABT FERMENTED MILKS

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This work is concerned with cultivation conditions (form of starter, inoculum, temperature and time of fermentation, enhanced non-fat milk dry matter or addition of whey protein concentrate) and their influence on quality of milk fermented by *Lactobacillus acidophilus* CCDM 151, *Bifidobacterium sp.* CCDM 94 and *Streptococcus thermophilus* CCDM 144. The tests were performed in sterile skimmed milk with addition of 2 % yeast extract for lactobacilli and bifidobacteria. Density of starter microorganisms, pH and concentration of L(+)-lactic acid, D(-)-lactic acid and acetic acid were evaluated. L(+)-lactic acid is easier metabolized in humans than D(-)-isomer, while the ratio of lactic acid and acetic acid influences antimicrobial and sensory properties of the fermented product. Organic acids were analyzed by electrophoresis and by the enzymatic method. The liquid non-concentrated starters provided higher density and lower pH after 16–18 hrs cultivation than the frozen ones. The higher the inoculum was (maximally 2.5% v/w) the higher the density and the higher the concentration of organic acids were achieved in bifidobacteria and lactobacilli while streptococci were unaffected. The inoculum of bifidobacteria had the only significant effect on the ratio of organic acids. When 1 % v/w used, the ratio of lactic acid and acetic acid was 3:2.6 and the ratio of L(+)-lactic acid and D(-)-lactic acid was 16:1. When 5% v/w used, ratios were 3:3.6 and 43:1, respectively. ABT milks should be fermented at 37 °C for 18 hrs. While Str. thermophilus provided appropriate pH below 4.6 within 14 hrs the probiotic cultures needed prolonged cultivation to achieve their maximal density. Addition of dried skimmed milk (with maximal effect at 12% w/w) enhanced growth of cultures and total amount of organic acids produced by means of the buffering effect and additional support of micronutrients, while whey protein concentrate was ineffective.

Keywords: *Optical isomers, lactic acid, LAB, fermentation profiles*

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G-7
IDENTIFICATION OF PROTEINS AND PEPTIDES IN MEAT AND BONE MEAL

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In Europe, the use of protein derived from animal tissues has been banned in livestock feed after the BSE (Bovine Spongiform Encephalopathy) epidemic. This prohibition requires the implementation of efficient means of controls. This research aims at developing protocols to determine the taxonomic and tissue origin of proteins present in meat and bone meal as feed for farmed animals. Peptidomics will focus on the proteic fraction of animal products or by-products by establishing their peptidic profile. With the help of enzymatic digestions that have been improved thanks to preliminary physico-chemical treatments of the sample, a diversity of peptidic fragments will be released. The global pattern as well as the identification of some of these peptides will provide markers for the determination of the origin of the detected animal proteins. Meat and bone meal (MBM) from different sources and from different animals (beef, pork, sheep, chicken…) was used as starting material. The MBM was first mechanically processed (with a grinder) before extraction with TCA/acetone. After purification (using a clean-up kit), the samples were resolved by 1D-electrophoresis on acrylamide gels. Selected bands were collected using a "spot picker" or cutting the gel by hand. The gel samples were treated with trypsin and the peptides were analyzed by mass spectrometry (maXis impact, Bruker) with the ProteinScape and Scaffold softwares. A gel-independent protocol has also been developed. This method has several advantages: cheaper, less-time consuming and easier to transfer in an industrial environment. The more abundant proteins are hemoglobin, collagen, beta-globin and keratin. A few species peptide markers have been found but they have to be confirmed. We hope by this approach to identify robust species peptide markers. In collaboration with the CER Groupe (Marloie, Belgium), antibodies will be raised against these peptides to develop routine ELISA assays. These new approaches to identify proteins and peptides in MBM would allow to reintroduce these by-products on trade and thereby to recycle slaughterhouse waste, providing cheap sources of protein to livestock.

Keywords: *Meat and Bone Meal – Peptidomics – Protein identification – Peptide biomarker – Mass Spectrometry*

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G-8
DISCOVERING UNEXPECTED CONTAMINATION: NONTARGETED ANALYSIS OF ORANGE JUICE AND MILK

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The increased globalisation of the food market and industry leads to an increased risk of food being contaminated by unexpected substances. Therefore, the Swedish National Food Agency and the Swedish Civil Contingencies Agency have started a project to create methods for detecting and identifying unexpected chemical hazards in food. Here we present a method using UHPLC–MS and untargeted analysis to identify the mass values and retention time of unknown contaminants. The samples were extracted with acetonitrile [1] and analysed with a gradient of 11%–100% methanol in water over 16 minutes [2]. The method has been tested in two blind studies, the first on orange juice and the second on milk. Sets of model contaminants were added to different samples, and compared to non-contaminated samples of different brands. The model contaminants were 18 pesticides in one set, seven mycotoxins in one set, and a pharmaceutical in the last set in orange juice and one set with 22 pesticides, a pharmaceutical and a colouring agent in milk. In both studies all spiked analytes were found. The concentrations were ranging from 0.4 to 100 ppm. In the light of the present results, we see a potential in developing this method for routine analysis of food on the market.

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Keywords: *Food safety, metabolomis, contamination, mass spectrometry, liquid chromatography*

GENERAL FOOD ANALYSIS

(H-1 – H-79)

H-1
THE PREVALENCE OF OVERWEIGHT AND OBESITY IN THE SOUTHERN AND WESTERN REGIONS OF KAZAKHSTAN

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Background. The obesity in the world is so widespread that the World Health Organization (WHO) has introduced a term called "obesity epidemic". Every 10 years the number of obese patients in the world increased by 10%. WHO predicts that by 2015, 2.3 billion adults will be overweight and 700 million will be obesity. In 1995 the national study of Kazakhstan revealed overweight or obesity at 42% of men and 47% of women. According to a study surveyed the nutritional and health status of the population of Kazakhstan in 2008, 50.6% of women and 45.4% of men had the weight above normal.
Objective. The study of the prevalence of overweight and obesity in the population of the southern and western regions of Kazakhstan.
Methods. The cross-sectional survey was conducted in October-December 2012. The sampling included 21 clusters, which involved the city, the district center and the village. Anthropometric measurements were conducted of all persons over 15 years of age living in the household, total n=1,647 people, including 1,089 women (n=732 in South, n=357 in West) and 558 men (the southern region of n=459, the western region of n=99). International standard for measuring the degree of excess fat deposits in the body was the anthropometric index - Body mass index (BMI).
Results. In the southern region, 51.6% of women had overweight (27.3%) or obesity (24.3%). In all 53.3% of men 38.2 % had overweight and 15.1% – obesity. The study revealed 63.3 % of women in West with overweight (35.0%) or obese (28.3%). In men, the prevalence of overweight (BMI≥25) was 54.6%, of which 26.3% of cases occur for overweight, 28.3% – for obesity.
Conclusion. Thus, it was found an increased risk of developing overweight and obesity in women than in men of the southern and western regions of Kazakhstan. This situation requires immediate communication for an active and comprehensive preventive measure among the population and to increase the knowledge of health workers on healthy intake, maintaining a healthy weight.

Keywords: *Overweight, obesity, body mass index (BMI)*

H-2
CHARACTERIZATION OF COMMERCIALLY AVAILABLE STEVIA-DERIVED SWEETENERS USING HIGH RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRY AND ADVANCED DATA PROCESSING

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Stevia is a sweetener derived from the stevia plant (*SteVia rebaudiana*). Stevia leaves contain diterpene glycosides, namely, stevioside, rebaudiosides A-F, steviolbioside, and dulcoside A, which are responsible for the typical sweet taste. Stevioside and rebaudioside A are the most abundant, accounting for 3–10% and 1% *w/w*. These compounds are 75 to 450 times sweeter than sucrose. Rebaudioside A (tetraglycoside) is known as possessing the best organoleptic properties while Stevioside (triglycoside) has a bitter aftertaste.The quality of the extract can be improved by utilizing glycosyltransferase enzymes to increase the glycosylation of the steviol-based components of the extract. This treatment decreases the bitter aftertaste of the extract, and increases the level of sweetness.The composition of the steviol glycosides also differ from region to region and plant variety.The ratio of the stevia components influences the quality and identity of commercially available stevia extracts.In this work, high resolution mass spectrometry in combination with advanced data processing was used for the characterization of different stevia sweeteners and dietary supplements.

Keywords: *Sweeteners, High-resolution Mass Spectrometry, TOF, Data Processing*

H-3
RAPID UHPLC ANALYSIS OF CAPSAICINOIDS IN DIFFERENT CHILI VARIETIES

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The consumption of chili peppers is generally associated with a pungent, burning or stinging sensation that is colloquially paraphrased as “hot taste”. However, if compared to the five primary tastes (sweet, sour, salty, bitter and umami), this very sensation might not be considered a “taste” at all, and in neurological terms rather reflects the perception of pain. The experience of pain is typically activated by potentially noxious stimuli mediators and is detected via specialized primary afferent sensory neurons termed nociceptors. Thus, the reason for the pungent feeling caused by irritants like e.g., capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide) can be explained on a molecular level by the interaction with special receptors (TRPV 1, or transient receptor potential vanilloid type 1) which again are also activated by thermal stimuli; hence the common perception of “heat” while eating chili containing foods. Besides the “classical” sens orial/organoleptic assay for quantifying “hotness”, nowadays analytics mainly emphasizes HPLC methods using various detectors while focusing on the determination of the individual capsaicinoids rather than on the overall pungency. Thus, the aim of this work was to establish a new rapid UHPLC method for the determination of capsaicin and dihydrocapsaicin as being the most powerful pungent capsaicinoids found in chili fruits and hot sauces (quantitatively as well as by pungency). Based on an implemented HPLC–UV method, gradient elution was adapted from a former 3.5 µm (100 mm × 2.1 mm i.d.) to a 1.7 µm (50 mm × 2.1 mm i.d.) UHPLC column, and further optimized to increase throughput while maintaining adequate resolution. Capitalizing UHPLC the net separation time could be reduce from originally 20 down to 1.7 min at a total cycle time of 4 min, hence presented a most feasible approach for high-throughput chromatography. Using UV (280 nm) and fluorescence detection (λEx 2 80 nm/λEm 310 nm), the established method was validated for the analysis of both capsaicinoids regarding linearity, precision, recovery and suitability of the sample preparation procedure. Compared to UV, fluorescence detection showed superior sensitivity with LODs ranging around 1 ng/mL, thus proved suitable for analyzing even very low pungent chili products. Moreover, to give an estimate on the massive variability between typical chilies, different varieties (cultivated in Austria) were characterized for their intrinsic pungency with total capsaicinoid amounts ranging from 200 µg/g for Jalapeño to 28000 µg/g for Bhut Jolokia corresponding to “mild” 3000 Scoville heat units (SHU) or “hot” 450,000 SHU. Despite this high variability as well as the partly complex matrix of some hot sauces, the established UHPLC method enabled reliable and interference-free capsaicinoid analysis throughout all samples, thus demonstrating a feasible tool for the high-throughput profil ing of the pungency of different chili products.

Keywords: *Chili, dihydrocapsaicin/capsaicin, UHPLC, HPLC*

H-4
HEALTH SAFETY OF MARGARINES TRADED IN POLAND

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In the recent years the population has been increasingly concerned with health related matters. Food, apart from supplying necessary nutrition components for the human organism, and providing sensual satisfaction, should be also of proper health quality and ensure health safety of consumers. The increase of interest in the so called “health food” of warranted quality was stimulated by the dynamic development of food industry, the creating of consumer market, growing competition and increasing consumer awareness and requirements. A very broad offer of plant fats in Poland allows consumers to choose from a wide range of products offered on the market. Consumers expect that hardened plant fats will not be harmful to health and provide proper nutrition qualities, desirability and sensual acceptance, reasonable price and availability of product. The composition of edible fats should – inasmuch as possible – comply with current nutrition recommendations, i ncluding those related to the content of saturated fat acids, trans-isomers and the level of metal elements content. The purpose of the study was the analysis of the composition of fat acids and the content of nickel and cadmium elements in 22 selected samples of popular cup margarines present on the Polish market and bought in retail shops. Fat acids were analysed in the form of methyl esters with the use of SRI 8610 gas chromatograph with Restek RTX-2330 column. As a result of the analysis acid profiles of margarines were obtained. The length of fat acid carbon chains ranged from C6:0 to C18:3, whilst C20:3 (cis-8,11,14) was found only in one sample in the amount of 0.31%. The contents of saturated acids varied between various kinds of margarine and ranged from 22.75% to 68.86%. C18:1 (trans-9) isomers were found in 13 samples and their content varied from 0.44% to 21.02%, whilst C18:2 (trans-9,12) isomers were found in two samples and their content varied from 0.28% to 0.32%. The content of metal elements in margarines was determined after their mineralisation in a Milestone Start D microwave with the use of 65% HNO_3 and 30% H_2O^2 (7:1 *v/v*) in 2000°C. The ASA atomic absorption method was used for this purpose. The contents of metal elements in analysed margarines varies in the following ranges: Ni – from 15.48 5 ppb to 264.640 ppb, Cd – from 0.000 to 15.545 ppb. The results show that the allowable threshold of the contamination with nickel (0.20 mg/kg in two samples) was exceeded.

Keywords: *Margarine,fat acids,metal elements*

H-5
THE ANALYSIS OF OXIDATION CHANGES IN PEANUT OIL, RESULTING FROM HEATING WITH MICROWAVES OF VARIOUS POWER

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The purpose of the research was the analysis of oxidation changes occurring in peanut oil while the oil was heated with 200W to 800W microwaves. The oxidation changes in oil, resulting from the heating process, were assessed in relation to the following values: peroxide value, anisidine value and the Totox value. The subject of analysis was the peanut refined oil manufactured by Oleificio Zucchi S.p.A., Italy, and bought in retail shops. The main components of the peanut oil are single saturated fats. The oil is characteristic of a mild taste and peanut fragrance. It is added to cold or hot meals. The heating of samples results in the rise of their temperature. The maximum temperatures of the oil depended on the power of microwaves applied. The highest temperatures were found to occur after 24–40 minutes of heating. The temperatures of the oil heated with 800 W microwaves ranged from 233°C to 235°C. Peroxide values increased over the period of heating. In samples heated with more powerful microwaves, i.e. 400 W, 600 W or 800 W, the maximum peroxide values were observed already after 6 to 12 minutes of heating. Hydrogen peroxides produced in the initial, up to 6-min. period of heating, were then decomposed into secondary products and heating, while continued, did not result in any further increase of peroxide values. A different mechanism of the production of hydrogen peroxides was observed in oil heated with 200 W microwaves, in which case the longer the heating period was, the higher the peroxide values occurred. In the oil heated with 400 W–800 W microwaves, the products of oil oxidation were produced within heating periods ranging from 6 to 12 min., which is proved by observed anisidine values ranging from 11.6 to 46.5. At the final stage of heating, the anisidine values were observed to drop. In the oil heated with 200 W microwaves the anisidine values were relatively lower and ranged from 6.4 to 15.1. The latter values were observed after heating periods of 24 min. to 40 min. Totox values, likewise anisidine values, increased over time and their variability curve was convex. In the oil heated with 600W microwaves, top Totox values were observed to range from 117.1 to 119.1 mEq O_2/kg after 30 to 40 minutes of heating. In the oil heated with 200 W microwaves, oxidation changes were relatively smaller. Totox value variability time curve was concave, i.e. the value increased up to 47.6 mEq O_2/kg after 40 minutes of heating.

Keywords: *Peanut oil, microwave heating, oxidative changes*

H-6
EFFECTS OF ESSENTIAL OIL *OF PIMPINELLA ANISUM* ON SENSORY PROPERTIES AND ANTIOXIDANT ACTIVITY OF YOGURT

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The purpose of this study was designed to investigate the effect of the addition of the essential oil of *Pimpinella anisum* on the quality and antioxidant capacity of the yogurt. The anise essential oil was prepared by steam distillation, and chemically characterised by gas chromatography-mass spectrometry (GC–MS) and determination of density.
The yogurt samples were prepared according to the standard method with different concentrations of the essential oil (0, 12.5, 25.0, 50.0 µL/100mL) and stored at 4±1°C. The chemical composition was determined after the first day. The survival of starter cultures *(Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophillus*) and viscosity, syneresis, color values, pH, acidity and antioxidant activity were monitored after 1, 7, 14 and 21 days. The yogurts with essential oil added had faster rates of pH reduction compared with plain yogurt. pH 4.6 was reached earliest for 50.00 µL/100mL –yogurt (140 min.) followed by 25.00 µL/100mL -, 12.50 µL/100mL - and plain-yogurts (260, 300 and 420 min. respectively). Viability of the starter culture was investigated during the storage of yoghurt at 4°C at the same time intervals. The results showed that the number of starter culture in all samples decreased during storage. Effect of the essential oil of Pimpinella anisum was not significantly different ($p<0.01$) from the control. The antioxidant capacity of plain-yogurt was unchanged throughout the storage period whereas all essential oil yogurts had higher ($p<0.05$) antioxidant activities than plain yogurt, both at the end of fermentation and throughout the storage period.
The results suggest that the essenyial oil of *Pimpinella anisum* may be used to modify the microbial fermentation of milk with the target of producing yogurt with higher antioxidant activity.

Keywords: *Yoghurt, antioxidant activity, esential oil, Pimpinella anisum*

H-7
BIOIMAGING OF RICE TISSUE WITH THE USE OF A LASER ABLATION SYSTEM COUPLED TO THE AGILENT 7700X ICP–MS

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The combination of a laser ablation (LA) system with ICP–MS has been developed over the last years for the direct analysis of solid samples with almost no sample preparation. More recently, this technique has been deployed to study the metal distribution in biological samples. Indeed, the analysis of tissue section using LA–ICP–MS enables the generation of pictures showing the element pattern in the sample. Therefore, it is possible to identify the localisation of the elements in the sample. In the present works, the application of LA–ICP–MS has been deployed on a rice grain. After the section of the sample, its surface has been ablated with a 213 nm wavelenght laser system. The abalation cell was directly connected to the Agilent 7700x ICP–MS for the elements analysis. Data were then converted into picture to create the element patterns present inside the rice sample.

Keywords: *Bio-imaging, ICP–MS, Laser*

H-8
METHOD DEVELOPMENT FOR THE DETERMINATION OF BIOGENIC AND VOLATILE AMINES IN FISH BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

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Biogenic and volatile amines have been used as chemical indicators for freshness in fish. A novel and simple method for the simultaneous determination of biogenic and volatile amines was developed without any additional derivatization step. Fourteen biogenic amines (Histamine, Spermine, Spermidine, Cadaverine, Putrescine, Agmatine, Citrulline, L-Ornithine, Tyramine, Tryptamine, 2-Phenylethylamine, Dopamine, Norepinephrine, Serotonin) and three volatile amines (Trimethylamine, Triethylamine, Tripropylamine) were determined using hydrophilic interaction liquid chromatography – tandem mass spectrometry (HILIC-MS/MS) in fish samples. The method steps are extraction, pH adjustment, filtration and analysis by HILIC–LC–MS/MS. Extraction was based on an official method for the determination of Total Volatile Basic Nitrogen, using 0.6 M perchloric acid. The extract was diluted with a buffer solution for pH adjustment (pH>3). Different pH adjustment solutions were stu died (CH_3COONH_4, $KHCO_3$, KOH) for the removal of pechlorates before LC–MS/MS analysis. For the chromatographic separation, a fused-core silica column (Phenomenex KINETEX, 2.6 µm, 100 × 2.10 mm) was used. HILIC parameters (mobile phase composition, buffer concentration, pH, temperature) were also optimized. The method was validated and applied in fresh and frozen fish samples.

Keywords: *Biogenic Amines, HILIC, LC–MS/MS*

H-9
A RAPID DNA EXTRACTION METHOD SUITABLE FOR ON-SITE APPLICATION

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Many crop plants have to be tested for genetic modifications when entering the European Union or when processed to food or feed derived products. The standard method for testing a sample for GMOs is to extract the DNA and then perform either a qualitative or quantitative PCR to detect and quantify any artificial inserts into the plant genome. Unfortunately, PCR requires high-end equipment and also skilled personnel. This drawback has been overcome by applying isothermal DNA amplification methods. Those simple procedures can be performed at constant temperatures of 37°C and 65°C and PCR thermocyclers are not needed anymore. However, for all those methods high quality DNA must be used as an input and the isolation and purification of DNA are still considered to be elaborate procedures. Among them, the most traditional one employs the surfactant cetyltrimethylammonium bromide (CTAB) and organic solvents, such as chloroform and isoamylalcohol for the dissolution of proteins while keeping the DNA in the aqueous phase. This method has been successfully used for this matter, as very reliable results can be obtained. Nevertheless, the entire extraction process is highly time-consuming as it can last between 3 to 4 hours and the amount of steps involved makes the procedure very tedious. In addition, it cannot be performed on-site as a centrifuge is always required. Other extraction methods based on commercial kits are also available for this purpose, but their price can be very high and the amount of sample which can be extracted is usually limited. The aim of this study was to develop a new method for extraction of DNA from maize that can be carried out on-site. Furthermore, the procedure should prove to be cheaper and simpler, but must perform equally as the more complex CTAB method. Numerous buffer systems were tested at different pH values to identify the optimal path for obtaining DNA in solution. In this case, the employ of a sodium phosphate buffer combined with enzymatic digestion of the matrix was determined to be the best performing procedure. Additionally, different filtration media were assessed to optimize DNA yield and purity.

Keywords: *DNA extraction, maize*

H-10
DEVELOPING A RAPID IN-FARM DIAGNOSTIC TEST FOR CAMPYLOBACTER

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Campylobacter is the most common cause of acute bacterial gastroenteritis in humans, causing around 600,000 confirmed cases of illness and around 100 deaths each year in the UK, and an estimated cost to the economy of £600M. Reduction of the incidence of *Campylobacter* in the food chain is a priority in the UK and in Europe, as part of a wider strategy to achieve a secure food chain. A major source of *Campylobacter* infection is poultry. Current systems to monitor infection in the farm involve sending samples to the laboratory, with the associated delays and costs. Therefore, rapid and robust tests are required that can be implemented on site and rapidly inform actions on the farm. This will enable farmers to react quickly and apply effective biosecurity measures, fulfilling an important need of the poultry industry. We are using a combination of DNA Loop-mediated isothermal amplification (LAMP) and antibody technologies to develop a rapid assay for detection of *Campylobacter jejuni* and *C. coli* in poultry faeces. Specific antibodies are used for bacteria isolation and concentration, precluding the need for bacterial culturing. Once the bacteria are concentrated on antibody-coated magnetic beads, they are transferred to a small, portable instrument where the LAMP reaction takes place, with fluorescence detection for assay readout. A kit is being designed containing all the consumables and reagents required for all steps, from sample preparation to results. We are aiming to reduce manual intervention to a minimum, thus minimising risks of contamination and allowing results to be obtained in about one hour from sample collection. We have developed a LAMP assay for *C. coli* and *C. jejuni* that shows specificity and high sensitivity in preliminary experiments. In addition, an immunocapture protocol has been developed for sample enrichment and clean-up. This step enables concentration of the bacteria present in the faeces sample (farm boot swabs) and elimination of materials and substances that might interfere with the DNA amplification reaction. A range of commercially available antibodies against C. coli and *C. jejuni* were screened for specificity and performance on immunocapture. Once the best performer was identified, the antibody binding protocol was optimised in turkey faeces (litter samples). We have shown that the bacteria isolated from the faeces can be transferred directly to the LAMP instrument, without the need for previous DNA extraction, and be detected in around 30 minutes. Current/future work include: a) optimisation of a LAMP internal amplification control, b) further specificity tests with a wide range of bacteria, c) determination of the limit of detection of the combined antibody/LAMP assay, d) final integration of the assay kit.

Keywords: *Campylobacter, LAMP, diagnostic, rapid test*

Acknowledgement: *Jeremy Hall (Bernard Matthews Ltd.), Terry Motley (Cranberry Foods Ltd), UK Technology Strategy Board*

H-11
ELEMENT SCREENING OF PINEAPPLE BY TOTAL REFLECTION X-RAY FLUORESCENCE (TXRF) SPECTROSCOPY

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The consumption of fruits and vegetables is part of a healthy nutrition and is recommended with a frequency of five times per day. In particular, the reduction of cardiovascular diseases is closely related to the consumption of fruits and vegetables. Therefore optimal nutritional composition of vitamins, secondary plant metabolites, minerals and trace elements are very important. Fruit juices, purees and other fruit containing products could provide an easy way to reach the uptake goal for fruits and vegetables. This work focused on pineapple, a tropical fruit usually consumed as fresh or canned, fruit or fruit juice, due to its distinct characteristic of containing minerals. The analysis was focused on the fresh fruit, where the distribution of manganese and other elements was determined. Samples of single-strength pineapple juice, of fruit juice concentrate and of puree from multiple origins were also analysed. A second aim of this work was to reveal the potential of TRXF as an alternative to other methods in metal determination. During this survey of the levels of transition metals in commercial fruit products an abnormally high level of manganese in pineapple juice was observed. Further investigation has revealed that pineapple appears to concentrate manganese to a much greater extent than other fruits. Levels of manganese (15±22 ppm) in commercial pineapple juices were found to be consistently higher than those of Cr, Fe, Ni or Cu and higher than in other common fruit juices. It was found that the manganese level in juices extracted from fresh Australian pineapples were variable, with some high and some low levels. Most of the manganese was found in the filtered juice and little in the pulp retained on a 0.4 micron filter. Electron paramagnetic resonance spectroscopy shows that most of the manganese is present as the $[Mn(H_2O)_6]^{2+}$ species. This means that a clarification of pineapple juice by fining agents and filtration cannot remove this manganese species. According to the literature, the high le vels of manganese probably reflect the acidic soil conditions in which pineapple is cultivated. The optimum pH value has been found to be between 4.5 and 5.5. High water content in soil leads to $Mn(II)^{2+}$ formation by MnO_2 (Pyrolusite) reduction by soil bacteria. The WHO recommendation for the tolerable daily intake (TDI) is 3.6 mg/day for adults (60 kg) with an upper limit (UL) of 11 mg. The toxicity of Mn as an essential element is regarded as low, but in higher concentrations it may lead to diseases as was found for industrial workers (neurodegenerative disorders like Alzheimer's disease, Parkinson's disease). In further studies the range of commercially available pineapple products will be extended. It is also planned to develop a fining method to lower the amount of Mn in pineapple juices.

Keywords: *Essential, element, spectroscopy, juice, manganese*

H-12
CUSTOMIZED AUTOMATED SOLUTIONS FOR FOOD LABORATORIES

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BIOTECON Diagnostics was founded in 1998 and is established as a well-known qualified global partner for the food and beverage industry as well as for producers of pharmaceuticals and cosmetics. BIOTECON Diagnostics focuses on development, production and marketing of rapid detection systems for pathogens, allergens, animal species, genetically modified plants and beverage spoilage organisms in food, feed and other matrices like environmental samples. The foodproof®, foodproof® SL and microproof® Detection and Quantification Kits, based on real-time PCR, are developed as easy-to-use systems to provide fast, safe and specific results for manufacturers and consumers. Customized automated Solutions: Now AOAC and MicroVal validated, BIOTECON Diagnostics has offered automated sample preparation and test setup solutions since 2009. The foodproof® RoboPrep+ Series, a fully automated system for the analysis of pathogens, was the first system on the market especially designed for the needs of the food industry. Automated sample preparation and PCR setup is the key to greater efficiency for the modern routine laboratory performing food analyses by PCR. Automation of sample preparation and PCR setup generates highly reproducible results and minimizes pipetting errors. foodproof® RoboPrep X-Tract powered by HTI performs automated sample preparation for labs with low to medium sample throughput. foodproof® RoboPrep+ powered by Xiril offers automated sample preparation and PCR setup in a food lab carrying out high-throughput pathogen analyses. A fully automated process with no manual handling steps is thus available for sample preparation and PCR setup of up to 96 samples in a single run. foodproof® RoboPrep Flex is compact and robust and has been validated for the detection of genetically modified organisms (GMOs), allergens and animal species. For these high-tech PCR automation platforms, a food sample preparation kit has been developed which is based on magnetic beads for DNA purification. The foodproof® Magnetic Preparation Kit III utilizes magnetic bead technology similar to BIOTECON Diagnostics' foodproof® Magnetic Preparation Kit I for Gram negative bacteria and the foodproof® Magnetic Preparation Kit II for Gram positive bacteria. This technology allows the generation of ultra-pure, ready to use genomic DNA for enhanced performance in sensitive downstream PCR applications. With the certification of MicroVal for the foodproof® RoboPrep® Series, BIOTECON Diagnostics is the first company that received ISO 16140 approval for a fully automated sample preparation worldwide. The validation by AOAC-RI was also completed successfully. For safer food – BIOTECON Diagnostics: simply builds up trust.

Keywords: *Real-time PCR, food analysis, pathogen detection, foodproof® Detection Kit*

H-13
STEC SCREENING AND STEC IDENTIFICATION USING REAL–TIME PCR

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Food-borne illness caused by Enterohaemorhagic *Escherichia coli* (EHEC) claimed 50 lives during one of the largest outbreaks in 2011. STEC have been encountered in leafy vegetables, sprouted seeds, raw milk and cheeses as well as fresh, minced and mixed meat preparations. Most infections have been caused by *E. coli* O 157. However, there are various other strains of non-O 157 *E. coli* capable of causing sickness and death that should be examined in food. Screening for pathogenic *E. coli* has been recommended by ISO using the virulence factors stx1, stx2 and the intimin eae gene. Serotype *E. coli* O104:H4, the cause of the severe outbreak in 2011, could have been detected if such screening had been in place. Used during the outbreack of 2011 by German government laboratories, BIOTECON Diagnostics developed the foodproof STEC Screening Kit to determine whether STEC are present in a food sample in less than 24 hours. This EHEC/STEC Screening is based on ISO/TS 13136, but was further designed to detect all variants of the stx1, stx 2 and eae genes. In particular, stx2f, which has been found in poultry, is missed using the ISO method, but easily using the foodproof STEC Screening Kit. Regarding to the CEN / ISO TS 13136 identification of O157, O26, O111, O103, O145 and O104:H4 are mandatory. This is also stated in EU regulation 209/2013 for STEC identification in sprouts. For the identification of 8 different serotypes (O26, O145, O103, O104, O111, O121, O145, O157), which also include the "BIG SIX", BIOTECON Diagnostics developed the foodproof STEC Identification Kit. All 8 serotypes can be detected in one single assay. These can be differentiated by using four channels. For safer food – BIOTECON Diagnostics: simply builds up trust.

Keywords: *Real-time PCR, rapid analysis in food, pathogen detection, EHEC, VTEC, STEC, foodproo® STEC Screening Kit, foodproof® STEC Identification Kit*

H-14
THE USE OF HYDROFLUORIC ACID FOR THE ANALYSIS OF ALUMINIUM IN IMPORTED NOODLES TO DETERMINE DIFFERENCES IN EXTRACTABLE AND TOTAL LEVELS OF ALUMINIUM

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The issue of elevated concentrations of aluminium in imported noodles was first discovered in Germany in November 2008 and confirmed by controls carried out by several other member states. From July 2012 to June 2013 there have been 19 RASFF notifications relating to aluminium in noodles, with concentrations found ranging from 13 to 82 mg kg^{-1}. Aluminium-containing food additives have been used in food processing for over a century, as a firming agent, raising agent, stabiliser and anticaking agent. As aluminium can also occur naturally, for example plants can take up aluminium from the soil and from water, a maximum limit of 10mg kg^{-1} has been suggested for ambient instant noodles with any higher concentrations presumed to be an indication of the presence of aluminium-containing additives. There are regulatory concerns that the tolerable weekly intake for aluminium is being exceeded by some groups of the population and it is therefore important that the concentration of aluminium in foods is accurately monitored. Methods are being assessed to establish if natural aluminium can be distinguished from aluminium added as an additive. One aspect of this concerns the use of hydrofluoric acid, HF, during microwave digestion as HF can dissolve naturally occurring aluminosilicates. Another aspect relates to distinguishing total aluminium from extractable aluminium. The measurement of the extracted aluminium is carried out by ICP–MS. Initial results demonstrate that using HF gave higher results compared with a digestion mixture without HF. However the ratio varies considerably from sample to sample. Further investigations are ongoing to determine if the increase in concentration when using HF is attributed to natural aluminosilicates or to other aluminium-containing additives.

MJ Walker Aluminum in Imported Noodles, 2011 http://www.governmentchemist.org.uk/dm_documents/Aluminium_Statement_DDRxT.pdf

Keywords: *Aluminum, noodles, extraction, ICP–MS*

H-15
FREE AMINO ACIDS VARIATION DURING SALAMI FERMENTATION

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A fast and reliable experimental protocol using gas-chromatography-mass spectrometry (GC/MS) was developed for analysis of free amino acids from dry fermented salami, applying [^{15}N]-methionine as an internal standard. The extraction procedure of free amino acids was followed by two-step derivatization procedure by esterification with butanol/HCl and trifluoroacetylation by using trifluoroacetic anhydride. Batches containing 0.3% glucono delta-lactone (GDL) and 0.05% sodium ascorbate (ASC), and batches containing only 0.1% sodium ascorbate were manufactured. The content of free amino acids was measured at different time intervals over 45 days of fermentation. It increased significantly with the fermentation time, especially in GDL variety. Glutamic acid (Glu), valine (Val), histidine (His) and alanine (Ala) were the most abundant, while cysteine (Cys) was generally low. The isotopic dilution GC/MS method developed here showed good validation parameters concerning linearity (r>0.98), RSD lower than 20% for precision and lower than 23% for accuracy, LOD bellow 1 ng. The method proved to be useful in fermentation process studies. It is suitable for routine analysis of low quantities of food samples and the results may be used for different purposes as: food quality control, food processing control, animals' diet control and metabolic studies.

Keywords: *Amino acids, dry fermented salami*

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H-16
ANALYSIS OF SENSORY PROPERTIES OF BEEG LEG BONE SOUP PREPARED BY DIFFERENT COOKING PROCEDURES

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The purpose of this study was to investigate the optimum cooking method of beef leg bone soup by varying pretreatment (soaking, blanching) and cooking (boiling) conditions. There are two different pretreatment, soaking and blanching, is a technique used to remove blood, off-flavor and impurities. For this purpose, sensory analysis was conducted.
The results obtained were as follows:
1. The first step in optimizing pretreatment, the soaking time was examined suitable by the redness in all the water extract from beef leg bone, then found out whether blanching should be conducted. As the soaking time increased, the redness of water extract from beef leg bone was increased ($p<0.05$) significantly during 6 hours. Especially, there was the greatest increasing at 2 hours soaking. However, it did not show any significant difference between soaking for 6 and 7 hours. We expected that soaking for 2 hours would be adequate for the convenience of cooking, if there is no significant difference in sensory properties between 2 and 6 hours soaking.
2. The second step in optimizing pretreatment, the factors affecting blanching was determined by sensory analysis. The flavor and overall quality significantly decreased ($p<0.05$). The sensory scores in flavor and overall quality showed that beef leg bone soup blanched in 10 minutes after soaking for 2 or 6 hours was higher than the other samples, but did not show significant difference statistically. These results suggested that soaking for 2 hours with 10 minutes blanching would be adequate, and do us good of cooking and saving times.
3. As the last step, the Korean traditional method that divide the total amount of beef leg bone with water into three parts and boil for 4 hours each, then put them together into one place, and the other method to boil all amounts of beef leg bone at once for 12 hours are compared. The beef leg bone soup which was pretreated by optimized pretreatment was compared for optimizing cooking method in terms of sensory analysis. Overall sensory scores were significantly increased ($p<0.01$) as cooking method. The preference in the all sensory characteristics was the highest in the beef leg bone soup pretreated by 2 hours of soaking and 10 minutes of blanching and boiled by the Korean traditional boiling method. It is turned out that boiling in the traditional way is the most desirable when producing soup with beef leg bone as a main ingredient.
The overall result of the study indicated that 2 hours in soaking and 10 minutes in blanching, then boiling in the Korean traditional method would be adequate for sensory properties. The optimized pretreatment and cooking method for beef leg bone soup will be necessary for the general use by cooks, and improve the level of productivity by saving the labor and cooking time.

Keywords: *Beef leg bone soup, pretreatment, cooking method, sensory analysis*

Acknowledgement: *This research was supported by HANWOO Board's 2013 grant.*

H-17
CHANGES IN THE RHEOLOGY AND FTIR SPECTRA OF SOME EDIBLE VEGETABLE OILS AS EFFECT OF THERMAL TREATMENT

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During the frying process, being in direct contact with oxygen and moisture and as result of high temperature (150–200ºC), various complex chemical reactions, such as thermoxidation, polymerization, fission and hydrolysis, take place in edible vegetable oils. This can induce an increase in their viscosity. The aim of this study is to assess the influence of duration of thermal treatment at 190°C on the rheological behavior of sunflower, rapeseed and palm oils. Because these oils have different contents of Saturated Fatty Acids, Mono Unsaturated Fatty Acids and Poly Unsaturated Fatty Acids we tried to establish if their different contents in fatty acids have influence on its rheology and FTIR spectroscopy. The content in unsaturated fatty acids influences the rheological characteristics of the studied edible oils, the viscosities measured at 25°C increase from 50.84 (2) mPa.s for original sunflower oil to 120.14 (6) mPa.s after 16 hours of thermal treatmen t. Rheological measurements being made at different temperatures from 5-70 or 80°C for sunflower and rapeseed oils and from 22.5–80°C for palm oils, an influence of their different contents in unsaturated fatty acids on the activation energy was observed. The duration of thermal treatment contribute towards the increase of the activation energy. The FTIR spectra of studied edible oils shows that the triglycerides, as major component in edible oils being dominant in the spectra. In the spectra obtained of the various samples, not all frequencies of the bands are exactly the same. The increase of trans disubstituted olefinic groups and the decrease of cis disubstituted olefinic groups could be explained by the isomerization of cis disubstituted olefinic groups as a consequence of thermal treatment of the oils, the variation of absorbance being influenced by the level of unsaturation of the oil.

Keywords: *Rheology, edible oils, thermal treatment, ftir spectra*

H-18
INFLUENCE OF DEEP-FAT FRYING PROCESS ON PHOSPHOLIPID MOLECULAR SPECIES COMPOSITION OF SARDINA PILCHARDUS FILLET

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Introduction. Fish is an excellent source of essential nutrients such as essential amino acids, bioactive fatty acids, minerals, vitamins, chitin and antioxidants. The nutritional benefit of fish lies, predominantly, in its lipid fraction which is mainly composed of phospholipids (PL) and triacylglycerols (TAG) exceptionally rich of n-3 polyunsaturated fatty acids (n-3 PUFA). Recently, fish PLs have attracted a great deal of attention as they are considered more efficient carriers of n-3 PUFA than fish TAG in terms of n-3 PUFA absorption in different tissues. In addition, fish PLs have also exhibited antitumoral and anti-inflammatory effects. Unfortunately, fish PLs are highly susceptible to lipid oxidation and to thermal damage due to excessive heating. The n-3 PUFA chains in PLs are the primary targets of oxidation which can take place during cooking processes. Since most fish are consumed cooked, the nutritional value of the final cooked product is of major importance for human health. Especially, the determination of the effects of frying (a very popular method utilized for fish cooking) on the n-3 PUFA rich lipid fraction of fish will provide useful information to consumers and to food industry to establish the fish quality.
Purpose. This study was, therefore, conducted to determine the influence of deep fat frying process on PL composition of edible muscle (fillet) of Sardina pilchardus, a fish species commonly consumed in Mediterranean countries.
Design/methodology. The effects of deep-fat frying performed using different culinary fats (extra virgin olive oil, conventional sunflower oil and high-oleic sunflower oil) and different frying temperatures (160 and 180°C) on the phosphatidylethanolamine (PE) and phosphatidylcholine (PC) molecular species composition (the preponderant fish phospholipid classes) were investigated. For each frying test, ten fish fillets were introduced into an deep fryer (capacity 2 L), in a closed environment, for 5 min. The oil temperature prior to start frying has been set to established value (160 or 180°C) and it was controlled by a specific digital thermometer. Each cooking procedure was done in triplicate. The PL molecular species composition was determined by high pressure liquid chromatography (HPLC) coupled with a second order mass spectrometer (MS-MS) with electronebulization interface (ESI).
Findings. The deep-fat frying process caused significative changes on PE and PC molecular species composition of the fish fillet. However, these changes were not related to the nature of the culinary fat and to the frying temperature. In all cases, the deep fat frying process caused a significative increase of the proportion of the PE and PC species formed by the combination of palmitic and docohexanoic acids and a significative decrease of the percentage of the PE and PC species formed by two docohexanoic acid residues.

Keywords: *Deep fat frying, European pilchard, phosphatidylcholine, phosphatidylethanolamine*

H-19
TESTING OF SILVER-CONTAINING PACKAGING MATERIALS AGAINST MEAT SPOILAGE RELATED BACTERIA

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Raw meat is an easily perishable product because of microbial activity and loss of sensory quality. Packaging has thus an essential role in preserving the quality and safety of the packaged meat. The need of extended shelf life and quality, developments in consumer behaviour and long transports set demands for new innovations for limiting bacterial growth in packaged raw meat. Antibacterial packaging materials have potential in preventing bacterial growth inside the package and thus improving product shelf life. In this study, silver is used as an active agent. Antibacterial effects of silver have been shown against various bacteria in vitro, whereas information about its effects in real food applications is limited. Packaging materials with silver were prepared by several methods. Selection of potential materials for meat packaging was based on in vitro testing against different spoilage-related bacteria. Shelf life of packaged meat was determined by examin ing microbial quality and sensory properties as a function of time.

Keywords: *Antibacterial, meat, packaging, shelf life, silver*

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H-20
MOLECULAR COMPOSITION OF THE NITROGEN FRACTION OF FOOD WASTE TO BE USED FOR FEED PRODUCTION

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Food processing activities in Europe produce large amounts of by-products and waste. Roughly one third of the food produced in the world for human consumption every year (approximately 1.3 billion Tons) gets lost or wasted, according to a FAO-commissioned study. Such wastes contain several reusable substances of biological value such as proteins, peptides and amino acids. Unfortunately they are only partially valorised at different value-added levels (such as spreading on land, animal feed or composting), whereas the main volumes are managed as waste of environmental concern. Direct disposal of such wastes to soil or landfill causes serious environmental problems because of their high biochemical oxygen demand. The most effective way of reducing the negative environmental impact is the incorporation of these waste streams into productive processes and gives them a surplus value by innovative technologies. In this scenario the European Project NOSHAN focuses to develop processes and technologies needed to use food waste for feed and feed additives production. Several food waste streams are considered and collected, selected according to their importance in food production in EU, their potential nutritional properties, quantities produ ced, the seasonality, the regulatory issues, the costs and the logistic of waste producers. A portfolio was created including wastes derived from selected fruits, vegetables, roots, tubers, dairy products, and cereals. In order to assess their potential to be exploited as raw materials for the formulation of functional feeds these waste streams were chemically analysed to fully characterize down to molecular level lipids, carbohydrates, fibers, nitrogen compounds, secondary metabolites. In this communication we will present an overview of the nitrogen fraction of these food waste. Total amino acids content was determined by LC/fluorescence in order to evaluate the biological values of proteins. The analysis of proteins by SDS PAGE, followed by MS identification of the protein bands after digestion, was a performed in the most protein-rich wastes in order to obtain information about the type of proteins present. Free amino acids were determined by LC/fluorescence in the most protein-rich compounds in order to determine the degree of proteolysis and because of their role as taste active compounds. Moreover peptides were also analysed by LC/MS in order to detect compounds with potential bioactive properties. Also the degree of amino acid racemisation was measured by GC technique, after hydrolysis and suitable derivatization, in order to assess the presence of D-amino acids either due to harsh technological treatments or fermentation. All these parameters were very useful to assess the quality of food wastes and their potential to be transformed in protein-rich feed or to use them as ingredient containing functional peptides.

Keywords: *Food waste, feed, amino acids, bioactive peptides, proteins.*

H-21
ANALYTICAL METHODOLOGY DEVELOPMENT AND UNCERTAINTY ESTIMATION FOR MOISTURE AND PROTEIN SCREENING BY NEAR INFRARED SPECTROMETRY IN CHICKEN BREAST MEAT

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This study aims to develop screening parameters through the employment of Near Infrared Spectroscopy (NIRS) technique using a FoodScanTM analyser, calibrated with Artificial Neural Networks (ANN) for Brazilian official inspection analysis of chicken meat. This method has been developed using as reference the classical determinations of moisture (gravimetric) and protein (Kjedahl titration) in broiler samples collected by the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA). The NIR technique is internationally used in industrial quality control. However, due to its inherent variations in methodology, is necessary to consider the uncertainty of the use of NIR for routine determination. In the study of chicken breast meat matrix, statistical analysis was performed indicating that no significant differences may be related to the time parameter, as the measurements were performed concurrently with grinding or one day after, with not notable changes in the analytical result. The use of two vials has given more robust results than just one. Calibration curves were performed using data obtained by NIR and the classic method for each parameter. They were determined by obtaining the correlation coefficients and performing statistical analysis (t-student and extended error). As the acceptance error ranges were obtained through the use of NIR technique as a screening method, it had its performance measured, and its uncertainty estimated. The results obtained for this matrix open the perspective of performing this screening method in other meat products.

Keywords: *NIR, Chicken Meat, Screening Method, Uncertainty Estimation*

Acknowledgement: *CNPq*

H-22
MONITORING OF NUTRIENTS CONTENTS ON NUTRITION LABELLING IN BREADS, SNACKS, NOODLES, DRINKS, PIZZA AND HAMBURGER IN KOREA

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Nutrition labels on food packages are designed to promote and protect public health by providing nutrition information so that consumers can make informed dietary choices. In our country, nutrition facts label was introduced as part of regulations in 1996, and became mandatory for most processed food products such as breads, snacks, drinks and child-favourite food that is sold in company which have more than 100 stores. The purposes of the study were to check the accuracy of the labeled amount. To do this, we analyzed calories, protein, crude fat, carbohydrate, sugar, sodium, fatty acid composition and cholesterol in breads, snacks, noodles, drinks, pizza and hamburger which the labeling is compulsory. The analytical methods basically followed the official analytical methods in Korean Food Code. In the most of the products, the measured value was consistent with the labeled value. But the measured values of some pizza and hamburger were more than 120% of the labeled values in sugar and sodium. The results of this study will be helpful to provide more reliable nutritional information to consumers.

Keywords: *Nutrition labelling, hamburger, pizza*

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H-23
INFLUENCE OF TECHNOLOGICAL PROCESSING AND OXYGEN SCAVENGERS ON SELECTED PARAMETERS OF PINEAPPLE JUICES

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Fresh pineapple juice represents important source of vitamins, minerals and/or phenolic compounds. Important factor influencing its quality and shelf-life is technological processing. Ascorbic acid and flavonoids concentration, as well as color stability and their antioxidant status are affected mostly by oxygen incorporated into juice during the processing. One of the approaches how to slow down these degradation processes and to prolong juices shelf life is the application of oxygen scavengers, i.e. materials incorporated into the package structure that effectively remove oxygen from the inner environment. Scavengers must be fast-acting high-capacity oxygen interceptors (if they should operate at the package gateway), capable of eliminating relatively large volume of oxygen. Few studies focused on quality of pineapple juices have been published, however there is hardly any information regarding the effects of PET bottle caps with oxygen scavengers on the q uality of pineapple juices, partly as due to the technology is rather new. Therefore the objective of this study was to assess the influence of different technologies in fresh pineapple juice processing, including the application of caps containing oxygen scavengers (O2S), on selected quality parameters of pineapple juice. Juice was prepared in two different ways. In the first, fruit juice was prepared by routine technology, and after the pasteurization was filled aseptically into PET bottles and closed by the standard cap SK38/16 (ST-C); while in the second, nitrogen atmosphere was involved in selected steps of juice processing, and bottles were closed by caps with oxygen scavenger SK38/16-O2S (O2S-C). Both types of juices were stored at 7°C in darkness during 91 days reflecting their declared durability. For the purposes of comparison of the effects of technology changes on juice quality, selected parameters, i.e., on ascorbic acid and total polyphenols concentration, as well as on changes in their antioxidant and radical-scavenging capacity and color stability were evaluated by means of HPLC, EPR and UV/Vis spectroscopy. Results obtained clearly confirmed that sample composition, technology of processing as well as storage conditions have significant effects on juice shelf life and its quality. New technology of juice processing connected with the application of screw O2S-C caps resulted in approx. 20% lower total color differences compared to these with ST-C caps. Although the decrease of ascorbic acid concentration revealed in both types of juices similar trend, in majority of cases, its concentration was slightly higher in juices with O2S-C caps. Antioxidant status of juices is also affected by the innovative technology, although in majority of assays used, the differences following from technology changes were recognized as minimal or ambiguous. Evaluation of kinetic parameters will give the definite answer on the partial topic of thei r radical-scavenging properties changes.

Keywords: *Pineapple juice, caps with oxygen scavenger, ascorbic acid, spectral characteristics*

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H-24
A NEW METHOD FOR MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM ORANGE

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Phenolic compounds are secondary metabolites of plants characterized by having in its structure at least one aromatic ring with one or more hydroxyl groups linked. Epidemiological studies indicate an inverse association between the intake of foods with high contents of phenolic compounds and the incidence of chronic diseases, such as cardiovascular disease, diabetes mellitus and cancer. Considering the importance of these compounds for health, it is important to quantify them in foods in order to identify the main sources. The microwave-assisted extraction (MAE) is a technique that has become popular for increasing the extraction yield in a shorter time, at the same temperature and using less solvent compared to conventional methods. In this work, we developed a new MAE method for extraction of phenolic compounds from orange [*Citrus sinensis* (L.) *Osbeck cv. Pera*]. The extractions were carried out in a Start-E microwave (Milestone) with the rotor SK-12 (12 cl osed vessels). A 23 face-centered central composite design (FCCCD) was performed with 18 experiments, including four replicates at the central point and six axial points. The following variables were evaluated: solvent volume, temperature and MeOH:water ratio. The dependent variable was the total phenolic content monitored using the Folin-Ciocalteau reagent. All variables were significant ($p < 0.05$), but their interactions were not, and the response surface model had a high coefficient of determination ($R^2 > 0.96$). The maximum value obtained in the FCCCD [62.97 mg GAE/100 g fresh weight (FW)] did not reach the value obtained by conventional extraction (71.64 mg GAE/100 g FW), which consisted of vortexing the sample, centrifuging and collecting the supernatant, repeating 5 times this procedure. The response surface model showed a positive effect of the solvent volume on phenolic compounds extraction, i.e., the higher the volume, the higher the extraction. Thus, a sequential o ptimization was performed by varying only the volume and maintaining the other variables at the optimum condition obtained by the previous experimental design. After the solvent volume optimization, the result obtained was 71.22 mg GAE/100 g FW. The method was successfully applied to both frozen and lyophilized samples. Furthermore, in this new MAE method was 70% faster, consumed 75% less solvent and used 50% less sample than the conventional method. In addition, up to 12 samples can be simultaneously extracted.

Keywords: *Microwave-assisted extraction, phenolic compounds, validation*

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H-25
CONTROL OF SALT CONTENT AND SODIUM REPLACER SALTS IN PROCESSED MEATS

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Processed meats typically contain salt in different amounts ranging from 2 to 7% of NaCl depending on the type of product and country. One of the products with the highest NaCl content is dry-cured ham that may reach large values within the range 5 to 7%. Due to current trend towards sodium reduction for better consumers´ health, several chloride salts from potassium, calcium and/or magnesium have been tested as potential replacers. The balance and final content for all these salts must be checked in different parts of the ham to assure the real sodium reduction levels as well as the regular distribution of salt replacers throughout the entire ham piece. The goal of this work was to develop a simple and easy methodology for the control of NaCl and also the salt replacers KCl, $CaCl_2$ and $MgCl_2$. The methodology to determine the content of cations and anions has been performed through water extraction followed by its analysis using ion chromatography with cond uctivity detection. The cations Na^+, K^+, Ca^{2+} and Mg2+ and anions Cl^- are easily extracted and correctly detected through such technique. This methodology allows an effective control of the salt content in processed meats but also a relatively easy way to verify the distribution and content of salt replacers.

Keywords: *Salt, salt replacer, potassium, processed meat, ham*

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H-26
MULTI-WALL NANOTUBES MODIFIED CARBON PASTE ELECTRODE AND PRE-TREATED PENCIL GRAPHITE ELECTRODE FOR ASCORBIC ACID DETERMINATION

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In this study, multi-wall carbon nanotubes (MWCNTs) immobilized on carbon paste electrode (CPE) and pencil graphite electrode (PGE) were used for ascorbic acid determination. It was found that MWCNTs concentration influenced the oxidation peak potential of AA. The lowest oxidation peak potential was found at the concentration of 2.0 mg/mL MWCNTs. It was observed that pH significantly influenced electrochemical behavior of MWCNTs/CPE since the oxidation peak potentials obtained in pH range from 7 to 10 were significantly lower than those obtained in acidic medium (pH 3.0, 4.0, 5.0, 6.0). Thereafter, amperometric detection (Britton-Robinson buffer pH 7.0; 400 rpm stirring speed; applied potential + 100 mV) was used in the case of MWCNTs/CPE exhibiting significant electrocatalytic activity towards ascorbic acid oxidation. The calibration curve was obtain in the range of 1.0×10^{-8} – 1.0×10^{-6} M with the limit of detection calculated to be 1.0×10-7 M of ascorbic acid. The pencil graphite electrode was pretreated prior the measurement applying potential +1.45 V for 60 s in 0.1 M phosphate buffer solution (pH 7.0) containing 0.1 M KCl. The influence of pH, potential and accumulation time was examined and the optimum conditions were found as: supporting electrolyte Britton Robinson buffer solution pH 3.0; constant applied potential -0.2 V; accumulation time 300 s. At these conditions, the calibration curve of AA was obtained in the range of $1x10^{-8}$ - 2.5×10^{-6} M. The precision of the method was investigated by repeated measurements of the 0.1 μM AA, and the relative standard deviation (RSD) was found to be 6.7%. The limit of detection was found to be 3.0×10^{-9} M. Comparing the both electrodes, pencil graphite seems to be the best choice since lower limit of detection was obtained and moreover, the electrode material is very cheap and requires no special handling and treatment.

Keywords: *Ascorbic acid, electrochemistry, carbon electrodes*

H-27
RAPID HONEY ANALYSIS AND CHEMIOMETRICS

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200 monofloral (acacia, chestnut, orange, linden, honeydew, sunflower, rhododendron) and multifloral honeys were analyzed with different techniques that do not require sample preparation (FTIR, NIR and electronic nose). Honeys were from a national competition selection and for each class at least 25 honeys were chosen. Chemometrics was applied to the single technique and to the three techniques put together with the aim to discriminate among the different botanical honey classes. Unsupervised cluster analysis techniques (PCA, dendrograms), classification supervised techniques (PLS-DA, LDA, SVM) and multiblocking techniques were used. For monofloral honeys though with the single analysis data elaboration it was possible to distinguish the different honey classes, multiblocking analysis (Consensus PCA) obtained the best results for both fitting and prediction. Difficulties were found in separating multifloral honeys from monofloral honeys in any way.

Keywords: *Honey, monofloral, multifloral, chemometrics*

H-28
SOURCES OF VARIABILITY IN THE ANALYSIS OF SPECIFIC PORK MEAT NUTRIENTS FOR FOOD COMPOSITION DATABASES

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Pork meat constitutes a food with relevant nutritional properties that can be found in many food composition databases even though the large natural variability in meat nutrients is not well reflected in such databases. In fact, the identification of the meat source is usually incomplete because only the animal species and type of cut, that may include several different muscles, are given. However, there are relevant nutrient substances in pork meat that are affected by intrinsic factors of pigs like genetics, age and type of muscle. For instance, the analysis of specific nutritional substances like carnosine, anserine, taurine, glutamine, coenzyme Q10, creatine and creatinine show a large dependence on the type of muscle. Meat cuts are usually composed of various skeletal muscles which contain various types of fibres of different metabolic type. The feed also exerts a relevant effect, not only in the amount of fat but also on its composition in fatty acids. All these sources of variability must be taken into account when including such data in composition databases because it may give very different values. This work shows the variability in the analysis of specific pork meat nutrients depending on the type of assayed meat and how they may affect the general food composition databases.

Keywords: *Nutrients content, food composition, variability, nutrients analysis, pork meat nutrients*

Acknowledgement: *Grant PROMETEO/2012/001 from Generalitat Valenciana (Spain) is acknowledged.*

H-29
EXTRACTION OF 2-ALKYCYCLOBUTANONES IN IRRADIATED MANGO AND PAPAYA USING ACETONITRILE. VALIDATION OF METHOD

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The 2-akylcyclobutanone marker is used for the identification of irradiated foods that it contain fatty. The official analytic method for the detection of irradiated foods it has been adopted by the European Economic Community (EN 1785:2003). The objective of this paper is to validate the method for the detection of 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) in irradiated fat-containing fruits using a quicker extraction method with acetonitrile and comparing it with the official shoxhlet standard. The results of this study showed the efficiency of the extraction method with acetonitrile for the detection of irradiated mango and papaya.

Keywords: *Food irradiation, radiolytic markers, 2-alkylcyclobutanone, acetonitrile.*

Acknowledgement: *I wish to express my gratefulness to all people that made possible this paper during the execution of the project ARCAL RLA5/060, as the International Organism of Atomic Energy (OIEA) and AENTA for the support and financing*

H-30
INFLUENCE OF THE DIETARY CAMELINA OIL ON THE CHOLESTEROL CONCENTRATION OF THE LONGISSUMUS DORSI AND SEMITENDINOSUS MUSCLE IN FATTENING PIGS

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The purpose of the study was to determine the Large White pigs treated with camelina oil have lower cholesterol concentrations two type of muscle. The experiment was conducted for a period of 63 days on 3 groups of Large White pigs (18 pigs/group) with an average initial weight of 40 kg/animal. In the end of the experiment, the 18 pigs were slaughtered and samples of Longissimus dorsi and Semitendinosus muscles were collected. The animals had free access to the feed and water. The control group (C) received a barley, wheat, peas, full fat soya and sunflower oil (3%) diet. The diets for the experimental groups (E1 and E2) had the same basal structure, but the sunflower oil was replaced by camelina oil (3%). Group E2 was also treated with a dietary antioxidant mixture (2%). The three diets had the same protein and energy levels (15.55% CP and 3192 kcal ME/kg. The fatty acids profile of the dietary oils and of the meat cholesterol was determined by gas chromato graphy using a Perkin-Elmer Gas-Chromatograph with flame ion detector (FID), using hydrogen as carrier gas. We used a capillary column BPX70 (DB-23 Length 60 m; Diam 0.250 mm; Film 0.25 um Agilent) for fatty acids and ELITE-5 (30 m, 0.32 mm ID, 0.1 um. df film) for cholesterol. The analytical methods were in agreement with SR CEN ISO/TS 17764-1 and 2: 2008 for the fatty acids, and with ISO 12228:1999 for cholesterol. Prior to the analysis, the fatty acids were transformed in methyl esters. For cholesterol determination, the fresh meat samples were saponified, extracted in petrol ether, concentrated and transferred on chloroform; the components were thereafter separated in the column and were identified by comparison with standard chromatograms. The fatty acids determinations from the sunflower oil have shown that the ratio of the polyunsaturated fatty acids ω-6 / ω-3 was 161.55, while the same determinations performed on the camelina oil produced a ratio of the polyunsatur ated fatty acids ω-6/ω-3 of just 0.63. The cholesterol concentrations from the Semitendinosus samples were 34.52±4.76 mg % in group E1 and 36.03±6.64 mg % in group E2, significantly (P≤0.05) lower than 46.99±8.71 mg % in the fresh sample from group C. The concentrations from the Longissimus dorsi samples were 30.17±4.98 mg % in group E1, 33.50±5.72 mg % in group E2, and 34.61±6.62 mg% in the fresh sample from group C. The higher concentration of ω-3 fatty acids from the camelina oil decreased the cholesterol level from the muscles samples collected from the experimental groups; the differences were significant (P≤0.05) compared to the control group for Semitendinosus muscle.

Keywords: *Cholesterol, fatty acids, gas-chromatography, pig, camelina oil*

Acknowledgement: *This work was Financed through the project PN 09380401*

H-31
MICROWAVE ASSISTED DIRECT TRANSESTERIFICATION OF TRIACYLGLYCEROLS FROM CHICKEN MEAT FOR FATTY ACID ANALYSIS BY GC–FID

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The quantitative study of lipids and fatty acids has been incorporated into the routine of many laboratories, and gas chromatography is one of the techniques used to determine the content and the composition of fatty acids in different samples. Many different methods of sample preparation can be carried out for gas chromatography analysis, and the most common procedure is lipid extraction followed by esterification of the fatty acids to form fatty acid methyl esters. However, this method is time consuming, expensive, depending on the amount and type of reagents, and it can lead to errors due to the multiple steps involved. Boron trifluoride-methanol has been used as catalyst for fatty acid esterification, and despite its effectiveness, it is costly and highly toxic. The use of microwave irradiation as an alternative energy source is already used in different chemical reactions. The aim of this study was to develop a microwave assisted transesterification of triacylglycerol method without previous lipid extraction and without the use of BF_3 for the analysis of chicken fillet fatty acids by GC–FID. A central composite rotatable design was used to optimize the procedure. Four different variables, i.e. sample mass, solvent volume, irradiation time and the concentration of NaOH solution in methanol, were used to evaluate the effects. The optimized variables were 300 mg of sample, 5 mL of 0.5% NaOH in methanol (*w/v*) and 35 min of irradiation. The new method was validated by comparison with the conventional method using BF3 and with a standard reference material of meat homogenate (SRM 1546, NIST). The fatty acid content obtained by microwave assisted direct transesterification did not differ from the content obtained by the conventional method or from the fatty acid content of the standard reference material. The microwave assisted direct transesterification method showed to be precise and accurate. Another advantage is that the tra nsesterification occurs directly in the sample, consequently, the lipid content is not necessary to calculate the fatty acid content by internal standardization. In addition, it allows the use of microwave energy to transesterify the triglycerides directly in the sample without the use of BF3, with advantages of low-time and low-cost.

Keywords: *Transesterification, basic catalysis, fatty acid, validation*

Acknowledgement: *CNPq*

H-32
THE DETERMINATION OF MAJOR AND TRACE ELEMENTS AND IODINE SPECIES IN MILK USING ICP–Q–MS

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Milk and milk products form a major part of a healthy diet. FDA recommended daily consumption of milk for children and adults is two and three cups daily respectively. As well as being a high source of calcium and potassium, milk contains a number of essential elements such as iron, copper and zinc. Multi-elemental analysis of milk can determine whether milk is meeting the expected nutritional requirements or is lacking either due to regional soil deficiencies in the case of cow milk or poor diet in the case of breastmilk. Alternatively, the anlaysis of trace elements that are toxic in nature can alert us to the possible risk of contamination in milk. As the sole source of nutrition for young babies and as a major constituent of the diet for older babies and toddlers, nutritionists are also eager to investigate the differences in bioavailability of some of the essential elements. As the bioavailability is dependent on the chemical form of the element, speciation analysis is perceived as the most promising approach to indentifying differences between baby formulas and breastmilk for example. The iCAP Q ICP–MS from Thermo Scientific was used for the analysis of cow milk, breastmilk and powdered baby formulas. New technologies introduced in the iCAP Q such as skimmer inserts for improved matrix tolerance and flatapole technology in the cell that enables full mass range KED analyses were exploited for the accurate and robust analysis of over 30 elements in the different milk samples. Speciation methodologies were also investigated for feasibility of determining certain components of the milk samples.

Keywords: *Milk, Iodine, Speciation, IC–ICP–MS*

H-33
DETERMINATION OF ALUMINIUM CONTENT IN BEVERAGE SAMPLES BY FLOW-BATCH SEQUENTIAL INJECTION SPECTROPHOTOMETRY

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A rapid, accurate and reliable flow-batch sequential injection spectrophotometric method for the determination of aluminium was developed. Eriochrome cyanine R (ECR) was used as a chromogenic reagent in the presence of N,N dodecyl trimetylammonium bromide (DTAB). The aluminium-ECR complex at pH 6 gave a maximum absorption at 584 nm. The sequential injection parameters that affected the signal response have been optimized. A linear relationship between peak height and concentration was obtained in a range of 0.0075–0.625 mg/L with limits of detection and quantitation of 0.0020 and 0.0070 mg/L, respectively. Relative standard deviations were 0.8 and 1.3% for 0.01 and 0.025 mg/L (n=11), respectively. The developed system was successfully applied for analysis of aluminium content in beverage samples. The results agreed well with those obtained from the ICP-AES method. The aluminium content in different type of containers was compared and evaluated.

Keywords: *Flow-batch analysis, Sequential injection, Spectrophotometry, Aluminium, Beverage*

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H-34
NUTRITIONAL PROFILE OF BALANITES AEGYPTIACA FLOWER

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Nutrient and antinutritional content of *Balanites aegyptiaca* flower was investigated; and found to have the following compositions; moisture content (43.3 ± 2.89%); ash content (6.67 ± 0.29%), crude lipid (4.5 ± 0.50%), crude protein (10.8 ± 0.49%) available carbohydrate (74.2 ± 0.49%), crude fibre (3.8 ± 0.29%), and calorific value (380.5 kcal/100g), Na (42.1 mg/100g), K (81.8 mg/100g), P (5.91 mg/100g), Ca (49.8 mg/100g), Mg (19.36 mg/100g), Mn (0.35 mg/100g), Fe (31.46 mg/100g), Cu (0.42 mg/100g), Zn (3.69 mg/100g), Cd (0.19 mg/100g), Co (0.33 mg/100g), Cr (0.35 mg/100g) and Ni (6.33 mg/100g). The *B. aegyptiaca* flower have sufficient amount of valine, and isoleucine. Moderate amount of leucine, methionine and threonine. Lysine is the most limiting amino acid in the flower. The concentration of antinutritive factors was observed to be phytate (1.63 mg), oxalate (0.15 mg), hydrocyanic acid (0.04 mg), saponin (4.67 mg), nitrate (0.02 mg) and alkaloid (28.7 mg); were lower than the reference toxic standard levels. Therefore, Balanites aegyptiaca flower could contribute in supplementing human nutrient requirement.

Keywords: *Nutrient, antinutrient, Balanites aegyptiaca flower, Edible wild plant*

H-35
DETERMINATION OF POLYPHENOLS IN CITRUS PEEL CAUSED BY GREEN MOLD (*PENICILLUIM DIGITATUM*) EMPLOYING LC–MS/MS

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Green mold is caused by the fungus Penicillium digitatum which is ubiquitous to all citrus growing regions. It is the most common and serious postharvest disease of citrus. The green mold induces the change of the flavonoid component distribution in citrus fruit. This studies on the role of these compounds in resistance to and on the resistance conferred by flavonoid in Citrus. It was confirmed by LC–MS/MS that as procedure of disease, while the content of hesperidin (HPD) increases, hesperetin (HPT) decreases. According to the literature Penicillium produces hesperidinase which hydrolyzes HPT to afford HPD.

Keywords: *Polyphenols, citrus peel, LC–MS/MS, Penicillium digitatum*

Acknowledgement: *This work was supported by a KBSI Grant (T33710) to J.S. Jin.*

H-36
SIMULTANEOUS DETERMINATION OF INORGANIC IODINE AND BROMINE SPECIES IN MUNICIPAL AND BOTTLED WATERS USING ANION EXCHANGE LIQUID CHROMATOGRAPHY COUPLED WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP–MS)

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A method based on IE-HPLC with online coupling to ICP–MS was developed for the detection of iodide, iodate, bromide and bromate in drinking water. Different combinations of flow rate and mobile phase concentration were evaluated to optimize the LC conditions for the elution profile of iodine and bromine species in terms of specificity and sensitivity seperately. Retention time, tailing factor, number of theoretical plates and selectivity factor alpha were measured to choose the best LC conditions. The optimal set up was determined as 32.5 mM concentration of mobile phase at 1.4 mL min^{-1} for iodine and 9.6 mM concentration of mobile phase at 1 mL min^{-1} for bromine species. The best LC condition for simultanious measurements of all four species was determined as 11.9 mM concentration of mobile phase and 1.2 mL min^{-1} flow rate. Limit of detection and limit of quantification were estimated as three and ten times the standard deviation plus the average of the blank, respectively. The LODs for iodide, iodate, bromide and bromate were 0.9, 0.4, 0.8 and 0.5 respectively. The regression coefficients (R^2) of all standard curves were better than 0.999 for all species. The repeatability of the method was evaluated, based on three replicate analysis at different concentration levels. RSD values for all four species were below 8% for all concentration levels. This method fulfills the requirements according to recommended values in standard methods with regard to the limit of detection and short time of analysis. The developed method was successfully applied to a range of water samples, including municipal, surface and bottled water. The municipal and surface waters only contained measurable amounts of bromide in low concentrations within the range of 7–11 µg L^{-1} that were similar between the different locations. The levels of other anions in comparison to some bottled mineral waters were lower. The bottled waters showed a high variation in halogen content. About two-thirds of the bottled waters contained bromide, with concentrations ranging between 3–1923 µg L^{-1}, a much larger variation than found in surface and municipal waters. The concentration of bromide detected in this study in some bottled mineral waters was relatively high and if ozonation or chloration is used for disinfection purposes in these cases, there can be a potential risk for the formation of bromate and trihalomethanes. Two of the bottled water samples contained bromate (2 and 4 µg L^{-1}), however below the upper allowable level set by WHO and the European Commission of 10 µg L^{-1} in drinking water. Bromate is possibly carcinogenic to humans and the admissible upper limit set by WHO and The European Commission is 10 µg L^{-1} in drinking water. Currently no legislation exists for iodide, iodate and bromide in drinking water in European countries and North America. However there are concerns on the toxic properties of iodate.

Keywords: *Iodine, bromine, drinking water*

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H-37
A NOVEL APPROACH FOR EVALUATING OIL STABILITY USING NON-THERMAL PLASMA TECHNOLOGY

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Today, food researchers are focusing on producing foods using innovative, healthier and more sustainable ingredients. This is especially true for lipids and fats, in recent years a trend has emerged in which unsaturated fatty acid levels in lipid-rich food matrices are being enhanced. These new lipid-rich food products, however, are increasingly prone to lipid oxidation during storage, resulting in the formation of unwanted flavours and aromas. Therefore, there is a growing request to continue research on the development and optimization of quality systems for the evaluation of lipid oxidation stability and corresponding flavour/aroma changes. For the determination of the oil stability, two different methods are widely accepted; namely the active oxygen method (AOM) and the Rancimat test. However, it is believed that such temperature based tests generally do not correlate sufficiently with the real shelf-life, or natural degradation of such food products, due to the widely divergent kinetics of lipid oxidation in function of the employed temperature (thermal degradation, polymerization and cyclization, or oxygen depletion). Moreover, some antioxidants are thermally instable, and hence their effect is often underestimated. Therefore, it is needed to develop accelerated lipid oxidation techniques at temperatures close to ambient conditions. In this work, three different methods for evaluating oil stability were compared using a commercially available vegetable oil blend as test matrix. Next to a naturally ageing and after Rancimat test, Non-Thermal Plasma Technology (NTP) was used for the first time as a highly innovative strategy for the determination of lipid oxidation stability. For this study an RF plasma jet was configured which generates reactive species that are capable of accelerating naturally occurring initiation and propagation processes in lipid oxidation. Headspace Solid-Phase Micro Extraction (HS-SPME) in combination with GC-MS was used for the identification and quantification of the most important lipid oxidation indicators in naturally aged oil. Chemical-analytical measurements on oil that was treated shortly with NTP, revealed that similar volatile secondary lipid oxidation products were produced as those in naturally aged oil (hexanal, heptanal, 2-heptenal, nonanal,…). These results indicate that this innovative technique has the potential to be an innovative and controllable tool to determine the oxidative stability in a much faster, objective and more realistic manner compared to the currently accepted techniques.

Keywords: *SPME, lipid oxidation, non-thermal plasma*

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H-38
MODIFYING QUECHERS FOR APPLICATIONS BEYOND FRUITS AND VEGETABLES

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Advances in modern instrumentation performance are accompanied by robust sample preparation techniques allowing for improvements in time and reduction in overall cost. The QuEChERS approach is an example of this evolution in sample preparation. The inherent flexibility in the QuECheRS procedure allows laboratories to tune the various steps to accommodate their needs in terms of matrix variability and analyte properties. However, while these optimizations can be as simple as exploring salt and sorbent variations, they can also include modifications that account for the sample pH, texture, and matrix complexity. The following work highlights applications where modification to the standard QuEChERS protocol was required, including pesticides in animal tissue and juice concentrates as well as analysis of botanicals and pet food additives. Together these applications will demonstrate how a simple method development approach can account for challenging samples and provide superior method performance.

Keywords: *QuEChERS, botanicals, juice concentrate, additive, sample preparation*

Acknowledgement: *Agilent sample preparation team, Jon Wong*

H-39
MONITORING OF ACETALDEHYDE IN ALCOHOLIC BEVERAGES

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Recently, concerns about the volatile hazardous compounds including acetaldehyde, methanol, and fusel oils in alcoholic beverages, which cause hangover such as headache and dizziness after consumption, have been raised. In the liver, the alcohol dehydrogenase oxidizes ethanol into acetaldehyde, which is then further oxidized into harmless acetic acid by acetaldehyde dehydrogenase. Acetaldehyde is carcinogenic in animal experiments and was classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) in 1999. In more recent years, acetaldehyde with alcohol consumption has been upgraded to Group 1, known as carcinogenic to humans. In the current study, acetaldehyde in alcoholic beverages was analyzed by GC–FID with capillary FFAP column after distillation. The quantitative analysis of acetaldehyde was performed with GC using the internal standard (butanol) method. We measured the acetaldehyde level in 140 al coholic beverages obtained from market including Soju and Cheongju. In Soju and Cheongju, each recovery rate of acetaldehyde was 90% and 91%, respectively. The LODs and LOQs were 1.6 mg/kg and 5.3 mg/kg, and 1.7 mg/kg and 5.7 mg/kg, respectively. All measured concentrations of acetaldehydes were far below the legally acceptable level of 700 mg/L.

Keywords: *Alcoholic beverages, volatile hazardous compounds, acetaldehyde, GC–FID*

H-40
TOXIC AND ESSENTIAL ELEMENTS IN VITAMIN AND MINERAL PREPARATIONS

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Macro- and microelements are essential elements needed to keep people in good condition and even life. They are supplied to human organism mainly with food but also with the increasing intake of different supplements. The latter are not only used in the cases of their deficiencies caused by inappropriate nutrition (i.e. restrictive diet, vegetarianism), illnesses (i.e. osteoporosis), physical activity (especially by athletes) but as an usual everyday habit. Every year on Polish pharmaceutical and food market a lot of different type of supplements appear. They are specially composed with vitamins and macro- and microelements which are dedicated to various groups of population and for different application with indication on pro-health action. These preparations are very often produced from natural sources as herbs and medicinal plants which apart from substances that may pay beneficial role to human health, may also contain contaminants as toxic metals that may be hazardous to human health. In 2010-2012 about a hundred vitamin and mineral preparations available on the Polish market, intended to use by different groups of population (children and toddlers, women, pregnant women, men, older people) were tested for the presence of macro- and microelements contents. Atomic absorption spectrometry methods based on FAAS, FES, ETAAS, VG-AAS techniques including quality assurance system were used in the study. Levels of the individual elements (beneficial and harmful to health) in the studied supplements varied considerably. The contents of macro-elements were as high as: calcium – 18%, magnesium – 32%, and potassium – 3% while the highest contents of microelements were as follows: zinc – 1.4%, copper and manganese – 0.2%, iron – 0.9%, chromium – 0.0007%, selenium – 0.016%. The contents of toxic metals as cadmium, lead and mercury were generally below maximum accepted levels (Commission Regulation (EC) No 629/2008).

Keywords: *Toxic metals, macro-elements, mict-elements, vitamin & mineral preparations*

H-41
COMPLETE AMINO ACID ANALYSIS OF FOODS AND FEEDS

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Amino acid analysis is used for the quantification of one group of essential nutrients in foods and feeds. To provide a robust, reproducible and accurate method for all the amino acids, both sample handling and chromatographic separation must be considered. Each sample must be hydrolyzed so that the amino acids are released from the proteins that comprise most of the available nutrients. Multiple hydrolysis approaches must be used because the amino acids are not all equally stable. Acid hydrolysis is used to determine the total protein composition. Acid hydrolysis following performic acid oxidation is required to measure sulfur containing amino acids. Alkaline hydrolysis is used to assess the amount of tryptophan. Microwave hydrolysis has been optimized for all three protocols to give improved control of hydrolysis conditions with better accuracy, reproducibility, speed and robustness. In this work we show the implementation of all three hydrolysis protocols for raw feeds, such as, soy bean meal, as well as complete mixed feeds. Chromatographic separation is used to identify and quantify the released amino acids. Reversed-phase UPLC® of amino acids derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl (AQC) provides high resolution and sensitivity. Linearity, precision, accuracy and robustness studies will be presented.

Keywords: *Amino, acid, uplc, solution, derivatisation*

H-42
DETECTION OF SOME PHENOLIC COMPOUNDS IN BARLEY AND RICE SHOOT RESIDUES USING DERIVATIVE SPECTROSCOPY

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The cereals are the most important sources of plant food for man and animals. This is not only true today, but it is being established since the earliest time. The true cereals are classified to be wheat, barley, rice, maize, oats and rye. Rice, barely, and wheat are the most important food cereals worldwide creating the daily source of nutrient for billions of people (Olofsdotter et al., 1997). On the other hand the importance of allelopathy in nature and agroecosystems has attracted researcher's attention with the main goal of using this phenomenon in biological control of weeds (Jones et al., 1999). Wheat, barley, and rice are the most important cereals in Iraq, where they are used for human food and livestock forage. Barley and wheat are cultivated in rainfed regions of the northern part of Iraq, while irrigated in the middle and the southern parts, thus, weeds cause great loss of yield that may reach 30–50% of total yield yearly. This study was aimed to investigate the allelopathic potential which might be found in local rice and barley cultivars. Most of the experiments were achieved in laboratory after optimizing all environmental factors to be suitable for growing crop plants and weeds. Second, soil type and texture were taken into consideration whether there may be any effect of soil porosity and particle size on allelochemical movement and action. Third to find out and evaluate the allelopathical magnitude of rice and barley residues as autotoxicity or as biocontrol for growing weeds through their decays. Time was insured for such decays to release various biochemics where two months were allowed before harvesting to plants in such soil amended with plant debris. The normal spectra of a mixture of various phenolic acids(vanillic, syringic, m-coumaric, p-coumaric, and p-hydroxybenzoic acids) and the aqueous extracts of shoots of Black barley, White barley, Aqra rice and Kasnadita rice. The comparison of the above curves showed that there were no characteristic peaks attributed to the presence of phenolic acids in the plant residue extracts. Therefore, the derivative spectra of the mentioned samples have been recorded. Derivative spectra have been used to identify the various phenolic acids present in the natural sample.

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Keywords: *Detection, phenolic compounds, barley and rice shoot residues, derivative spectroscopy.*

H-43
ASSESSING MICROBIAL DIVERSITY CHANGES DURING COCOA BEANS FERMENTATION

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The first stage in chocolate production is a microbial fermentation of cocoa beans. Several groups of microorganisms are responsible of numerous biochemical and physical changes inside and outside the beans. Classical microbiological methods fail to detect most microbial species. Therefore, molecular methods, such as Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis (PCR–DGGE) fingerprinting was utilized to assess the microbial ecology of cocoa fermentation. In this study the microbial ecology of wood box fermentation from Ivory Coast was investigated using 16S rDNA and 26S rDNA to detect variations in the ecology structure of bacteria and yeasts, and populations of individual species were determined at three time intervals by sequencing of DNA purified from DGGE gels. Analyses of DGGE patterns show that cocoa beans fermentation generated great variations in the microbial ecology in terms of number of species and their relative abundance. The bacterial ecology quickly undergoes dramatical changes during the process after two days of fermentation the dominance of Enterobacteria, first with Lactic acid bacteria and at the lastest stages by acetic acid bacteria. Furthermore, the yeast *Hanseniaspora opuntiae* predominated during cocoa bean fermentation as its abundance remain stable throughout the whole process. Other yeast species showed great variations in abundance along the process (arise or disappear).

Keywords: *PCR-DGGE, Fermentation, Cocoa beans, Microbial ecology.*

H-44
EFFECT OF REFINING ON THE PHYSICOCHEMICAL PROPERTIES OF CASTOR SEED OIL

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Oil sample was extracted from Castor seed using soxhlet extractor with *n*-hexane as refluxing solvent. Thereafter, some portion of the oil was purified by degumming; neutralization and bleaching. Some physicochemical parameters of the crude and refined oil were analyzed. These include: Moisture content (13.00±1.41% and 15.00±1.41%), specific gravity (0.92±0.0002 g/ml and 0.93±0.0002 g/ml), pH value (8.65/25°C and 9.35/25°C), saponification value (183.26±1.62mgKOH/g and 176.72±2.81 mgKOH/g), free fatty acid value (0.85±0.00% and 0.56±0.03%), iodine value (12.69±0.00I2/100 g oil and 10.47±1.341l2/100g of oil), ester value 181.38mgKOH/g and 171.47mgKOH/g), acid value (0.94±0.32mgKOH/g and 0.84±0.28mgKOH/g) and peroxide value (1.85±0.07 meq and 1.70±0.00 meq) for crude and refined oils respectively. The result of the analysis shows that there is good improvement in the quality of the oil after refining, demonstrated by a decrease in those characteristics that determine stability notably free fatty acids and peroxide value. Similarly, the result showed that refining did not have much effect on the iodine value, moisture content and specific gravity. Hence, the oil is of good quality and could be recommended for industrial usage.

Keywords: *Castor, seed, oil, extraction, refining.*

H-45
RESIDUAL DETERMINATION OF LACTOSE IN FOODS AND COMPLEX MATRICES BY LC–MS/MS

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Lactose intolerance (lactase deficiency or hypolactasia) is when the body does not produce enough lactase to break down lactose, a sugar found in milk and many other milk derived dairy products and foods. The reduction of lactase activity after infancy is a genetically programmed event and for this reason the majority of the Earth's population is lactose intolerant. Therefore, consumers and food industry show increasing interest in lactose free foods and related certifications. A simple and fast method for extraction and purification steps of lactose in food matrices has been developed through proper changes and adaptations of procedures previously reported in scientific literature. By applying LC–MS/MS, lactose was detected and it was obtained a chromatographic resolution capable of separating alpha-lactose/beta-lactose/alpha-maltose/beta-maltose and almost all of the disaccharides. It was operated in positive mode to detect lactose molecules (as $[M+Na]^+$ sodium adducts) and it was used labeled $^{13}C(12)$-lactose to get the maximum possible control to qualify (relative retention time) and to quantify the analyte (matrix effect and precision). There is, however, a small percentage of real situations (difficult samples) in which only chemical approach and resolution (both chromatographic that spectrometric) are not sufficient in terms of selectivity and sensitivity. In these cases biochemical approach with the selective enzymatic procedure together with the LC–MS/MS are able to solve the problem of determination residual of lactose in food matrices.

Keywords: *LC–MS/MS; resolution; lactose; residual; intolerance*

H-46
CRITICAL POINT: THE PROBLEMS ASSOCIATED WITH THE VARIETY OF CRITERIA TO QUANTIFY THE ANTIOXIDANT ACTIVITY

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An antioxidant is a compound that decelerates the oxidation of other chemicals, which provides a protection to the important cell components by neutralizing natural free radicals that are typically formed during cell metabolism. Free radicals are highly reactive compounds, that interact with the nearest stable molecules (proteins, lipids, carbohydrates and DNA), taking its electron and transforming them in a free radical, beginning a chain reaction, that may result in damage for the surrounding living cells. Free radicals may be either oxygen derived (ROS) or nitrogen derived (RNS). An important factor that may counteract their effect is the alimentary intake of antioxidants. Hence the interest, since two decades ago, for evaluating the antioxidant activities present in all types of foods and characterizing their specific mechanisms.
At present there is no a convenient assay that enables the evaluation of the antioxidant capacity in a food system, mainly because factors affecting oxidation reactions and antioxidant activities differ. The methods to test the antioxidant capacity have still leaved many open questions. The in vitro assays can only rank the activity for their particular reaction system and their relevance to in vivo health protective activities is uncertain. However, the common practice of choosing between 3 to 6 assays for each food component does not obey usually to mechanistic considerations, but to the attempt to minimize the problem of the variability of the results by differences in the matrix of the component studied, the oxidizable substrate, the oxidizing agent, the antioxidant used as control, the characteristics of the system (aqueous, lipidic, multiphasic) and variables such as temperature and pH. Thus, it is logical that in the last decade, researchers have claimed unity of the approaches and have tended to standardize the protocols to increase the method effectiveness for in vitro and in vivo responses. In addition, the tendency to accumulate data rapidly has support authors to use simple calculation formalisms, forcing the conditions of the assay to assume a linear kinetic response, in which samples are generally assessed using a single time and single dose. Very often the same method is performed with different experimental protocols and formalisms for quantifying the activity. This has caused an important loss of information and the risk of erroneous conclusions.
In this work we propose unity of the operative requirements corresponding to the accomplished oxidation reaction of all the particular problems associated with the diverse quantification criteria used for most typical methods. By collecting data from the bibliography, the problems of using single-time quantification procedures, disregarding kinetic considerations, are discussed in detail. Furthermore, those criteria that takes into account the kinetic of the process (dose-time-dependent behaviour) are also evaluated and compared.

Keywords: *Antioxidant activity; antioxidant methods; mathematical modelling; kinetic response, toxicological issues.*

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H-47
QUANTIFICATION OF ANTIOXIDANTS IN MODEL FOOD EMULSIONS

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The effectiveness of antioxidants (AO) in emulsions is determined by both the rate constants and the concentration of the AO at the reaction site, which is believed to be in the vicinity of the interfacial region. The rate constants and the concentration of AOs at the reaction site depend on their chemical structures and are affected by the nature of the emulsion components (oil and emulsifier). Therefore, these factors must be taken into account when selecting or designing antioxidants for a particular application. However, predicting the distribution of an AO in emulsions is much more complex than in binary water-oil systems. In fact, the addition of emulsifiers to prepare kinetically stable emulsions creates an interfacial region, and therefore, two partition constants are needed to describe AO distribution, the one between the oil-interfacial and one between the aqueous-interfacial regions. Numerous attempts have been made to measure AO distributions in model food emulsions, primarily by isolating the oil and water regions followed by determining the AO concentration in each region by employing different analytical tools. This approach, however, does not provide information on the percentage of AO in the interface because the emulsion is broken previously to any analytical measurement. Thus, determining the AO distributions in emulsified systems needs to be done in the intact emulsion system. In this work, a kinetic method that does not require isolation of phases was employed to estimate the partition constants of AO between the oil, interfacial and water phase. This method [1] is grounded in the pseudophase model for thermodynamically stable microemulsions and exploits the reaction of 4-hexadecylbenzenediazonium ions, 16-ArN2+, trapped at the interfacial region of the emulsion, with antioxidants. The observed rate constant (kobs) of the reaction is monitored by employing a derivatization method based on trapping unreacted arenodiazonium ion as an azo dye. A large serie of dihydroxycinnamates of C1–C16 fatty acids with increasing lipophilicity were synthesized. The percentage of compounds in the interphase increased progressively with increasing chain length up to a critical point, beyond which the percentage of the compounds decreased. The C8 ester was the compound with the highest concentration at the interface region. The results are relevant for interpreting the effects of lipophilization of antioxidants on their efficiencies. The chemical kinetic method should work with a wide range of AOs [2] provided they react with the – and a range other emulsion components—provided they do not react with ArN2+.

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Keywords: *Emulsions, antioxidants, partition constants, distribution*

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H-48
RAPID ANALYSIS OF GLUCOSE, FRUCTOSE AND SUCROSE CONTENTS OF COMMERCIAL SOFT DRINKS USING RAMAN SPECTROSCOPY

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The objective of this study was to quantify of glucose, fructose and sucrose in commercial soft drinks by Raman spectroscopy as a fast and low cost technique. For the calibration, dilutions that were in the range of % 0-12 (w/w) were prepared for each of glucose, fructose and sucrose. The Raman spectrum of each dilution was obtained. Partial least squares (PLS) regression method was used to carry out the spectroscopic data analysis. Calibration models were formed and curves were plotted with full spectrum Raman data that were taken from these dilutions. The contents of the sugars in the soft drinks were predicted depending upon the calibration models by PLS. The slope of regression values of glucose, fructose and sucrose were 0.967, 0.992 and 1.008 and the coefficient of determination (R^2) values were 0.913, 0.998 and 0, 993 for validation, respectively. The high pressure liquid chromatography (HPLC) method was used to verify the efficiency of the Raman method. The coefficient of determination values between the HPLC and predicted values of glucose, fructose and sucrose were determined as 0.913, 0.968 and 0.908, respectively. The result of this work provides a rapid method for evaluating the quantitatif analysis of glucose, fructose and sucrose in soft drinks.

Keywords: *Raman Spectroscopy, Glucose, Sucrose, Fructose, Chemometry*

H-49
CHARACTERIZATION OF INFANT FORMULA QUALITY PARAMETERS USING THE FAST METHOD AND THE AMALTHEYS® ANALYZER

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Infant formulas' composition is strictly regulated at European level. Following the 2008 melamin-adulteration scandal in China, monitoring of infant formulas quality parameters has become of paramount importance for industrials and final consumers. A set of 4 quality indicators based on fluorescence was developed to answer the need for rapid and non-destructive assessment of IF quality parameters, including the rehydration ability of the powder and the protein nutritional quality. These indicators are measured using the Amaltheys fluorescence analyzer. The Amaltheys analyzer proposes to assess whey soluble and denatured proteins and extent of Maillard reaction in 5 min, according to the patented FAST method. The principle of the method is to prepare a soluble supernatant at pH 4.6 containing the pure soluble whey proteins and measure protein autofluorescence resulting from native tryptophan and neoformed advanced Maillard products. The first fluorescence is measured at excitation/emission 280/340 nm and is strongly correlated to the Kjeldahl non caseic nitrogen fraction; the second one at 340/430 nm is mainly correlated with carboxymethyllysine. Calibration and validation of the measurement is achieved using certified whey standard and reference material. We evidenced that Amaltheys enables accurate assessment of whey protein denaturation rate and Maillard reaction products, with good correlation to conventional indicators. The amount of denatured whey proteins is obtained by substracting the soluble whey proteins to the total whey content in the recipe. We show that the level of denatured whey protein is the main predictor for powder solubility and dispersibility. The FAST index was indicative of the heat charge applied during the process in relation to the extent of the Maillard reaction. The FAST index was well correlated with furosine and CML in the case of high lactose content. However, oxidation of LC–PUFA in the presence of transition metal ions had a stronger impact on the FAST index, especially when lactose concentration was low. A benchmarking study was performed to evaluate the potential of the FAST method to discriminate 24 commercial powdered infant formulas according to their quality parameters. The three quality indicators measured with the Amaltheys analyzer, soluble, denatured whey proteins and the FAST index, were coupled to another fluorescence signal, particle scattering at 280 nm, to discriminate and classify the formulas using PCA. We demonstrate that the combination of the four fluorescence signals allows discriminating three main classes of infant formulas according to their nutritional properties and rehydration parameters.

Keywords: *Infant formula, fluorescence, protein denaturation, Maillard reaction, principal component analysis*

H-50
APPLICATION OF SOURCE-INDUCED FRAGMENTATION OF CHONDROITIN SULFATE AND HYALURONIC ACID BY LC–MS

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Glycosaminoglycans (GAGs) are anionic polysaccharides consisting of repeating disaccharide units. These compounds are difficult molecules for analysis by mass spectrometry due to their complexity in saccharide composition, polydispersity, and sequence heterogeneity. In this study, a liquid chromatography coupled to direct electrospray ionization mass spectrometry (LC/ESI–MS) and tandem mass spectrometry (MS/MS) analyses performed in negative ion mode, is described for the analysis of chondroitin sulfate (CS), consists of repeating units of [GlcAβ(1-3)GalNAcβ(1-4)] and hyaluronic acid (HA), an unsulfated GAG consists of repeating units of [GlcAβ(1-3)GlcNAcβ(1-4)].). GAGs readily undergo source-induced fragmentation when analyzed by electrospray mass spectrometry with the use of high source cone voltage. At the dissociation cone voltage (defined as minimum cone voltage to dissociate all polymeric molecules), CS and HA produced simple mass spectra consisti ng primarily of monosaccharide and disaccharide ions derived from glycosidic bond cleavages. This chemistry enables the collective detection of a polysaccharide through the detection of one or more small saccharides. Subsequently, this method was applied for analyses of chicken cartilage hydrolysates and products of their ethanol precipitation to assess the quality and suitability of these materials for possible production of joint food supplements. And also this method was used for determination of CS and HA content in selected joint food supplements, available on the market in the Czech Republic.

Keywords: *Chondroitin sulfate, hyaluronic acid, LC–MS, source-induced fragmentation*

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H-51
ANALYTICAL STRATEGY FOR DETERMINATION OF INULIN IN JERUSALEM ARTICHOKE

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The Jerusalem artichoke (*Helianthus tuberosus* L.) tube is a vegetable with a low caloric value and store carbohydrates in the form of inulin instead of starch. The inulin is a lineary polymer of D-fructose joined by β(2→1) linkages and terminated with a D-glucose molecule linked to fructose by an α(1→2) bond, as in sucrose. Inulin and oligofructose are a significant part of the daily diet of most of the world's population. They are functional food ingredients which after a unique combination of nutritional properties and important technological benefits. In food formulations, inulin and oligofructose may significantly improve organoleptic characteristics. Other plants are containing inulin include leeks, onions, garlic, asparagus, dahlia, yacon and chicory. Within this study a method for the determination of inulin using the Ultra-Performance Liquid Chromatography coupled with Evaporative Light Scattering Detector (UPLC–ELSD) was developed and optimized. The analytical method is based on determination of the inulin pursuant the amount of resulting monosacchatides fructose and glucose after acid hydrolysis. The limit of quantification (LOQ) of saccharides is 0.05 mg mL^{-1}. Finally, the content of inulin in technological-step process to obtaining inulin from the tubers of Jerusalem artichoke was examined.

Keywords: *Inulin, oligofructose, UPLC–ELSD, Jerusalem artichoke*

Acknowledgement: *The financial support of the Technology Agency of the Czech Republic, project No TE01020080, Centre of competence for bio-refining research.*

H-52
LS-NAA FOR STUDYING THE REPRESENTATIVENESS OF DOG FOOD SAMPLES

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Large sample neutron activation analysis (LS–NAA) allows non-destructive analysis of bulk samples, providing the measurement of several chemical elements. Analysis of kilogram-sized samples can be especially advantageous when homogenization is not feasible or when large objects cannot be damaged for sampling purposes. Dog food is a complex material, composed of several different ingredients added for supplying all the required nutrients. Therefore, taking small samples representative of an entire production lot can be a quite critical step. In this work, LS-NAA and conventional NAA were applied to study the representativeness of small test portions (250 mg) of dog food collected from regular 1 kg packed bags. The measurements allowed to assess the within bag and between bag variation of chemical elements in dog food. Twenty-four bags of dry dog food of one specific brand (1 kg each) from the same production lot were acquired in a pet-shop in Delft, The Netherlands. The entire content of each bag was packed into a polyethylene bottle and analyzed using the large sample NAA facilities at the Hoger Onderwijs Reactor of the Delft University of Technology, The Netherlands. Following particle size reduction in a knife mill, one test portion of 250 mg from each of the 24 bags was packed in polyethylene vials for conventional NAA at CENA, after irradiation in the IEA-R1 reactor, IPEN/CNEN, São Paulo, Brazil. The elements Br, Ca, K, Na and Zn could be measured by both LS-NAA and conventional NAA. Except for K, the standard deviation of the mass fractions in the 24 large samples was higher than can be explained from the uncertainty of measurement, indicating differences of composition amongst the bags. However, only for Zn the variation can be considered somehow relevant (rstd = 15.5%), while for Br, Ca, K and Na the relative standard deviations, which included both the measurement uncertainty and some degree of difference between bags, were lower than 6%. For conventional NAA, Zn also presented the highest variation (r_{std} = 14.6%) amongst the 24 small samples, while Br, Ca, K and Na presented relative standard deviation lower than 5%. The variation of results obtained from conventional NAA agreed well with that from LS–NAA, confirming the representativeness of the small portions for measuring the five chemical elements, since it is quite a critical step the sample size reduction for chemical analysis, i.e. 250 mg portion for a bag of 1 kg.

Keywords: *LS–NAA, dog food, chemical elements, representativeness*

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H-53
CHARACTERIZATION OF MELANOIDINS FROM THE MAILLARD – PHENOLIC COMPOUND INTERACTIONS IN MODEL SYSTEMS

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Various dietary phenolic compounds are reported to influence the nonenzymatic browning development by altering the flavour profile of thermally processed foods and contributing to the formation of novel high molecular weight Maillard-type products of diverse technological and physiological properties. In order to gain more insight in the heat-induced Maillard – phenolic compound interactions, light to dark brown melanoidins were isolated from heated (125°C, 120 min) mixtures of amino acids (L-(+)-lysine, L-(+)-arginine or glycine), D-(+)-glucose and ferulic or caffeic acid, with or without lipid oxidation products (hexanal or (2*E*)-hexenal). SPME–GC–MS analysis of the thermal decomposition (250°C, 10 min) products, alkaline hydrolysis and antioxidant activity measurements, employing DPPH, FRAP and Folin-Ciocalteu's assays, were combined to characterize water-soluble and nonsoluble fractions obtained. Model reaction products were additionally compared with the most widely analysed dietary melanoidins, isolated from coffee (100 % *Coffea arabica,* medium roasted) brews.
The obtained results indicated that the presence of phenolic compounds altered the browning capacity and thermal degradation profile of model melanoidins by favouring the release of volatile benzene derivatives (e.g., 2-methoxyphenol, 4-ethyl-2-methoxyphenol, 2-methoxy-4-vinylphenol, acetovanillone, 1,3-dimethylbenzene, catechol and 4-ethylcatechol) in the headspace of heated samples. A considerable share of the primary ferulic acid degradation product 2-methoxy-4-vinylphenol (up to 92% of the total GC peak area) indicated that hydroxycinnamic acids could be incorporated as intact molecules into model melanoidins. After alkaline hydrolysis, 44–3952 µg of ferulic acid and 4 – 140 µg of caffeic acid were found for 100 mg of water-soluble high molecular weight (HMW, > 12 kDa) fraction, respectively. The recoveries of both hydroxycinnamic acids were the highest for amino acid, glucose and hydroxycinnamic acid model systems, followed by the oxidized lipid-containing ones and, finally, coffee brew melanoidins. Corresponding trends were observed comparing the composition of different model systems and the percentage of 2-methoxy-4-vinylphenol in the headspace of thermally destructed melanoidins. The radical scavenging activity and reducing power of model melanoidins increased more than two times when phenolic compounds were added to lysine and carbonyl compound mixtures. Maillard reaction products from heated arginine, glucose and ferulic or caffeic acid mixtures showed similar antioxidant capacity as coffee brew melanoidins. HMW products comprised the major part of the water-soluble antioxidants from the Maillard – phenolic compound interactions in model systems.

Keywords: *Maillard reaction; phenolic compounds; melanoidins; thermal degradation; antioxidant activity.*

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H-54
USE OF MULTIVARIATE STATISTICS TECHNIQUES FOR THE SEPARATION OF SEVENTEEN CAPSINOIDS BY UPLC

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Fruits of chili pepper plants belong to the family Solanaceae and genus Capsicum which are one of the most consumed spices throughout the world and are very important commercially. The consumption of chili peppers is due mainly to their pungent flavour. The pungency is caused by capsaicinoids, but recently,a new family of non-pungent compounds similar to capsaicinoids was found, the capsinoids. This family of compounds has the organic function ester in place of amide and this difference cause the pungence absence, however, the capsinoids have similar biological activities to capsaicinoids such as antioxidant, anti-inflamatory, antimicrobial, antimutagenic and promote the weight loss and decrease the fat body accumulation. This work shows the use of multivariate statistics techniques for the development of a separation method of capsinoids by UPLC. Capsiate, dihydrocapsiate and other 15 minor capsinoids were synthesized and their structures were confirmed by NMRH1. An UPLC (ACQUITY UPLC H-Class, Waters) equipped with an ACQUITY UPLC Photodiode Array Detector was used and two column (Waters Acquity UPLC BEH C18 column; 2.1 mm I.D.; 1.7 µm particle size; 50 and 100 mm of length respectively) were tested. A mobile phase composed by water and acetonitrile (CAN), both solvents with 0.1% of acetic acid, was used. A central composite design and response surface as design of this experiment, with three variables was used: First: Initial percentage of acetonitrile; Second: Gradient (linear) time change until 100% of acetonitrile and third: the flow rate. The response chosen was the resolution into each pair of peaks and the time analysis. A multi-criteria response technique of Derringer and Suich was used, and the desirability values were established for each individual response and they were combined into their recommended global desirability function, with the objective of minimize the analysis time keeping a good separation between the compounds. The best condition found for the column of 100mm was composed by an initial mobile phase with 41.84% of ACN, changed to 100% of ACN in 3.96 min. and a flow rate of 0.679 mL min^{-1}. For the column of 50 mm the best condition found was composed by 50% of ACN of initial mobile phase, changing to 100% of ACN in 7.72 min. and a flow rate of 0.8 mL min^{-1}. In both conditions a good separation was found, showing the applicability of the statistical tools for the capsinoids analysis. Finally, the column of 100 mm had a better relation "separation-time analysis".

Keywords: *Rapid Method, UPLC, Capsinoids, Multivariate Statistics*

Acknowledgement: *FAPESP, CAPES, CNPQ, UNICAMP, UCA*

H-55
USE OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TOF-MS DETECTION TO ANALYSE A,A-TREHALOSE AND OTHER DISACCHARIDES IN PRUNUS AND MALUS FRUIT GENERA

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α,α-Trehalose is a disaccharide that is considered to have important properties as abiotic stress biomarker [1] and conservation agent [2]. Until now, the presence of this compound has been described in some fruits like peach (Prunus persica). However, its determination is difficult due to the chromatographic complexity of the chromatogram area where trehalose appears together with other disaccharides, which have very similar structures. Given the importance of α,α-trehalose, a GC×GC–ToFMS method has been developed using a 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a Pegasus 4D ToFMS (LECO Corp., St. Joseph, MI, USA) to analize this compound and other disaccharides eluting at the same chromatographic area. The use of bidimensional chromatography is almost mandatory in this case because it provides higher peak capacity and lower detection limits [3], which allows to separate and quantitate this family of compounds that present major problems in conventional 1D gas chromatography. Samples analyzed included peach, apricot (*Prunus armeniaca*) and apple (*Malus domestica*) where endogenous content of trehalose is reported by the first time. Extraction of metabolites was carried out with a polar solvent mixture consisting of water and methanol. A derivatization step including methoximation and silylation was requiered in order to increase the the vapour pressure of the compounds and m ake them suitable for a gas chromatography analysis. Furthermore, palatinose (6-O-α-D-glucopyranosyl-D-fructose), a disaccharide which is not naturally found in fruit samples was used as internal standard. Finally, statistical analysis was carried out to distinguish disaccharides composition among fruits and varieties.

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Keywords: *α,α-Trehalose, disaccharides, GC×GC–TOFMS, fruits*
Acknowledgement:

H-56
ULTRAPERFORMANCE CONVERGENCE CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY: A NEW WAY FOR DETERMINATION OF CHOLESTEROL IN LIPIDS INCLUDING HUMAN ADIPOSE TISSUE

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Cholesterol represents a substance commonly found in animal fats, tissues, blood, bile, and cell membranes. In addition, many important compounds are formed from cholesterol (e.g., steroid hormones – corticosteroids, sex hormones, vitamin D). Last but not least, cholesterol contributes to the formation of bile acids which are essential for the absorption of fat from the diet. Although cholesterol belongs to essential compounds for the human organism, this depends on the amount and form present in the human body. For instance, a high level of total cholesterol in blood represents a risk factor for atherosclerosis. In medical laboratories, the concentration of the total cholesterol in human serum is commonly determined by spectrophotometric techniques. Since in humans cholesterol is present mainly in the bound form as esters of fatty acids (70%) and less in the free form (30%), enzymatic hydrolysis is typically conducted prior the spectrophotometric determination. In this study a method for the determination of free and ester-bound cholesterol in human adipose tissue (washing tissue, abdominal tissue) employing a novel ultraperformance convergence chromatography (UPC2) coupled to a high-resolution mass spectrometer with a quadrupole/time-of-flight analyzer (QTOFMS), was developed. The results were obtained by a procedure consisting of Soxhlet extraction of adipose tissue using a mixture of hexane-acetone (3:1, *v/v*). Extracted lipids were re-dissolved in isooctane and analyzed using UPC2–QTOFMS operating under the conditions of atmospheric pressure chemical ionization (APCI). Using this approach comprehensive lipidomic profiles of adipose tissues were obtained. On the basis diagnostic fragment ion m/z 369 representing protonated molecule of dehydrated cholesterol, the peaks of free and bound cholesterol were identified. The data obtained by UPC2–APC–QTOFMS were correlated with the results obtained by spectrophotometric method. A good correlation between these two methods for 23 samples was obtained for both serum and adipose tissue.

Keywords: *UPC2, cholesterol, lipidomic, human adipose tissue*

Acknowledgement: *Financial Support from Specific University Research (MSMT No. 20/2013).*

H-57
DETERMINATION OF FATTY ACIDS IN ALGAE AND SIMILAR LOW-FAT MATRICES

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The interest of consumers to improve their health by eating the "healthy fats" continues to increase, hence using of dietary supplements as a source of omega-3-fats becomes increasingly common. As an interesting alternative source of these healthy omega-fats, some strains of freshwater micro-algae can pose a great challenge. Currently, an extensive research focused on cultivation of various micro-algae strains in order to increase the yields of poly-unsaturated fatty acids in the algae biomass is being in progress (BIORAF project). For the purpose of reliable quantitation of fatty acids in such "difficult" matrices, reliable analytical method had to be developed. Determination of fatty acids in the lyophilized algae matrix is rather complicated from two reasons: algae contain fairly low content of fat (maximum 15%), and the quantity of the sample provided for analysis is typically very low (0.1–0.5 g). Hence, the analytical method has to be sensitive enough to be able to detect such low fatty acids concentrations. During our research we developed the analytical micro-scale method allowing the direct quantification of fatty acids in the sample without the necessity to determine the overall fat content. The method involves extraction of lipids from the lyophilized homogenous algae sample with the dichlormethane–methanol mixture, after addition of the nonadecanoic acid as a surrogate. After the extraction step, co-extracted pigments are removed by using of the active charcoal. The methylation procedure takes place in a reaction cup with reduced amount of the reaction agents. For separation, gas chromatograph HP 6890 Series (Agilent Technologies, USA) equipped with a polar column SP-2560 (100 m × 0.25 mm; 0.20 μm) was used, for detection, the flame-ionization detector (FID) was enabled.

Keywords: *Fatty acids, FAME, microalgae, gas chromatography*

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H-58
CHANGES OF VOLATILE PROFILES IN STORED POULTRY MEAT

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Meat, a worldwide consumed food commodity, possesses a specific aroma that undergoes various changes during so called meat aging and, as well, during long term storage. The changes start immediately after the animal slaughtering and involve chemical, biochemical and microbial processes. These changes can have a serious impact not only on sensory quality, but, as well, on the overall hygienic-toxicological quality of meat. In our study we were examining the possibility to differentiate between fresh and stored meat by measuring the profiles of volatile compounds. The measurements were carried out by the technique of solid phase microextraction combined with gas chromatographic separation and mass-spectrometric detection using time of flight mass analyser (SPME–GC/TOFMS). Volatile profiles of raw chicken and turkey meat showed differences in the spectra and intensities of compounds during storage (up to 168 hours) under common refrigeration conditions (4°C). Besides the originally present substances, the products of microbial activity such as ethanol appeared in stored meat. Based on statistical evaluation of acquired volatile profiles it was possible to group the samples according to the storage time. In the case of smoked turkey meat the changes in volatile profiles were not so pronounced during 168 h storage. No products of microbial activity were identified in accordance with the expected preserving effect of smoking. On the other hand several compounds originating from smoking fluid (furan-2-carbaldehyde and 1-hydroxypropan-2-on) and from wood combustion (phenol, 4-methylphenol, 2-methoxyphenol), were identified, mainly on the surface of the meat.

Keywords: *Volatile compounds, chicken and turkey meat, storage of meat*

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H-59
PREPARATION OF FATTY ACID METHYL ESTERS – COLLABORATIVE TRIAL

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The aim of the trial was to evaluate and validate each of the 4 derivitisation procedures defined within BS EN ISO 12966-2:2011 for the preparation of fatty acid methyl esters. Oils and fats (i.e. liquid and solid lipid) are predominantly composed of fatty acid esters of glycerol (triacylglycerols, TAGs), with smaller amounts of fatty acid esters of sterols and long chain aliphatic alcohols. Due to the high molecular weight of the TAGs and their consequent low volatility, they are difficult to analyse directly by gas chromatography (GC), especially if a detailed analysis of unsaturated fatty acids is required. Fatty acids themselves do not chromatograph well (except for short chain length fatty acids, e.g. butyric and valeric acids). It is therefore better practice to form fatty acid esters, usually the fatty acid methyl esters (FAMEs), prior to GC. The analysis of oils and fats has been extensively reviewed the in scientific literature. The formation of FAMEs is a critical stage in the analysis. Non-quantitative conversion of fatty acids to FAMEs, modification of the structure of fatty acids (e.g. changes in positional and geometric isomers present) and formation of non-FAME artefacts may all affect the quantitative determination of fatty acid composition. BS EN ISO 12966-2:2011 contains guidance on the preparation of fatty acid methyl esters prior to GC–FID analysis. In support of this guidance various procedures to prepare fatty acid methyl esters are detailed. These include: - a 'rapid' transmethylation procedure under alkaline conditions; - a 'general' transmethylation/methylation procedure under sequential alkaline and acid conditions; – a BF3 transmethylation procedure; and, - an alternative procedure using acid-catalysed transmethylation of glycerides. Collaborative trial participants were asked to analyse 6 different oil samples as blind-duplicates using each of the four derivatisation procedures defined within BS EN ISO 12966-2:2011 followed by GC–FID analysis. For 12 samples, 48 derivatisations and analyses were therefore undertaken by each laboratory. Once derivatisations were complete participants were asked to split each derivitised sample into 2 portions. Participants analysed one portion using their own in-house GC–FID procedure for a minimum of 24 marker FAMEs (as well as MUFA, PUFA, etc..) and send the second portion to a central laboratory who analysed all participants samples for the same marker FAMEs. Analyses undertaken by the central laboratory will minimise between-laboratory effects enabling the performance of each derivatisation procedure to be evaluated using a selection of non-parametric statistics.

Keywords: *FAMEs, GC–FID, collaborative trial, oils, derivatisation*

H-60
HIGH-PRESSURE HOMOGENIZATION OF POLYSACCHARIDES ASSESSED BY AF4

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Polysaccharides, in particular cellulose, starch, alginate and κ-carrageenanes are important biomolecules used as additives in the food industry. Most foods are emulsions with usually structured water and oil phases made of mixed ingredients and additives. Starch has been used for decades and is still added as a thickening agent by increasing the viscosity (gelation effect), like in ice-cream or in margarine. The knowledge about the true composition and molecular structure, in particular molecular weight and branching of these additives will influence the final product properties (emulsion stability). The impact of modifications thereof by processing and treatment has been addressed recently [1–4]. Field-Flow Fractionation (FFF) invented in 1966 now has become a powerful separation tool based on chromatographic principles in the range of 1kDa to 1012 kDa. The separation is achieved within flow streams in an unpacked channel, which is beneficial for even sens itive samples since no shear degradation and filtration effects, like in SEC take place. A flow stream force perpendicular to the sample stream line ensures fractionation of the sample according to the hydrodynamic volume of the analytes in asymmetrical Flow Field-Flow-Fractionation (AF4). The present work is intended to support any user dealing with detailed analysis of high and low molecular weight biomolecules. In particular, the poster focuses the effect of low and high pressure homogenization on the molecular structure (Mw) of polysaccharides. AF4 coupled to suitable detectors (MALLS, UV) proofed to be the method of choice to get a deeper insight into polysaccharide modification by detection of large Mw up to 106 kDa down to very low Mw of 10 kDa thus giving a whole picture of the resulting treatment process.

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Keywords: *Polysacharides,cellulose, starch, Field-Flow Fractionation, AF4*

H-61
USE OF DIFFERENT HILIC STATIONARY PHASES FOR THE CHROMATOGRAPHIC RETENTION OF MEAT POLAR COMPOUNDS

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In HILIC, mobile phase forms a water-rich layer on the surface of the highly polar stationary phase creating a liquid-liquid extraction system. The analytes possessing higher polarity have a higher affinity to the stationary aqueous layer than the analytes possessing weaker polarity and so they show higher retention times in the chromatographic separation. In this way, mobile phase pH influences on polarity by affecting ionisation of analytes, and thus their retention characteristics. A total of four columns with four different polar stationary phases (bare silica, amide and zwitterionic groups bonded to both porous silica, and polymeric beads) were selected to study the effect of mobile phase pH on retention characteristics of some polar compounds typically found in meat. The results showed that the mobile phase pH has a significant impact on retention and selectivity in HILIC by influencing solute ionisation. The pH was adjusted to 3.5, 6.0, 7.8, and 9 to manipulate selectivity. In all silica based columns, pH 3.5 offered the worst selectivity for the compounds under study. These conditions on polymeric column results in excessive retention, so higher pH conditions were necessary in order to elute the analytes. Neutral pH values (pH 6.0 and pH 7.8) offered good retention times and kept the best resolution for all compounds. Retention times in all columns remained essentially unchanged at pH 6 and pH 7.8. Polymeric column was also tested at pH 9 and also no differences in retention were observed. Acid mobile phase pH conditions mainly charge basic and phosphorylated groups, whereas both acid and basic groups are charged at higher pH values. At low pH conditions the retention of basic compounds such as carnosine and anserine was higher than in neutral/basic pH conditions in which these compounds eluted earlier. In summary, HILIC chromatography is a reliable method to separate meat compounds. Polarity and ionisability characteristics of these compounds at different pH conditions together with stationary phase interactions influence on retention behaviour of all compounds under study. Chromatographic conditions tested at pH values above 6.0 show good results in all columns.

Keywords: *HILIC; mobile phase pH, stationary phase; meat polar compounds; meat markers*

Acknowledgement: *Grant PROMETEO/2012/001 from Generalitat Valenciana (Spain) is acknowledged.*

H-62
DEVELOPMENT OF RAPID METHOD FOR DETERMINATION OF ENDOPHYTIC PHYTOHORMONES – SUBSTANCES FOR AGRICULTURAL PLANTS GROWTH REGULATION

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Phytohormones represent a group of low molecular substances involved in the coordination of various physiological processes in plants. Their presence in relative low concentrations is related to promoting of plant growth and increasing of plant resistance to environmental factors. Besides their natural occurrence in plants, the production of many phytohormones with identical structure and comparable biological function was proved also by various endophytic microorganisms, including both bacteria and fungi. A rapid method for simultaneous determination of nine phytohormones in microbial culture media was developed and optimized. The spectrum of analytes, namely indole-3-acetic acid, gibberellic acid, gibberellin A1, trans-zeatin, dihydrozeatin, N6-isopentenyladenine, trans-zeatin riboside, dihydrozeatin riboside and N6-isopentenyladenosine, covers the major groups of plant growth regulators. During the method development and optimization, following parameters were assessed: *(i)* ionization efficiency and detection ability obtained by using three different mass-spectrometric systems (UHPLC–MS/MS, Qtrap 5500, AB Sciex; UHPLC–MS/MS, Xevo TQ-S, Waters; UHPLC–HRMS, Exactive, Thermo Scientific), *(ii)* mobile phases for chromatographic separation and the injection solvent selection, and *(iii)* the sample preparation strategy. When analysing very low-concentrated compounds, as the phytohormones are, the detection sensitivity and quantification limits achievable are of the greatest importance. From these terms of view, Qtrap 5500 was assessed as the most promising. From several injection solvents examined, water showed to be the best choice regarding to the chromatographic peaks shape under the reverse-phase separation and aqueous ammonium formate / methanol as the mobile phases. Regarding the sample preparation, homogenization of microbial biomass by using the ultra turrax in order to release the possible intra-cellular endophytes into the aqueous / buffered medium was performed. By using of the above mentioned sample preparation, separation, and detection conditions, concentrations as low as 0.1–2.5 ng/mL was possible to quantify in the raw, microbial culture medium (of course, homogenization, centrifugation and microfiltration were performed before the sample injection). This high throughput approach developed offers a simple way for screening of phytohormones produced by microbial endophytes.

Keywords: *Phytohormones, plant growth regulators, endophytes, UHPLC-MS/MS*

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H-63
PREPARATION OF A COLOSTRUM DERIVED BOVINE IGG CERTIFIED REFERENCE MATERIAL

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Bovine colostrum is a high value product of the dairy industry. It is a popular dietary supplement worldwide that is marketed to support the human immune system. Colostrum is obtained from cow's milk collected in the first few days after giving birth and manufactured into a powder. Colostrum products are assessed and marketed based upon their IgG content, and New Zealand colostrum has an international reputation for high quality and purity. Currently, the most common methods of evaluating the IgG content of colostrum powders are based on Protein-G affinity chromatography or immunological techniques. An uncertified IgG standard derived from bovine serum is generally used for calibration and this raises some technical and QC issues as to comparability and traceability of results. Measurement Standards Laboratory of New Zealand (MSL) collaborated with Cawthron Institute, Nelson to investigate the need for an IgG certified reference material (CRM) derived from bovine colostrum, developed a process to purify IgG from bovine colostrum, and validated analytical methods to assess the IgG content of fractions during the purification process. A very pure IgG CRM has been prepared from bovine colostrum (96% monomeric IgG, 4% dimer) and investigated the stability during storage in a range of formulation buffers. This presentation discusses the need, purification issues, analytical assessment tools and stability data from the studies.

Keywords: *Colostrum, IgG, reference material*

Acknowledgement: *Cawthron Institute*

H-64
THE FOOD SAFETY RESEARCH INFORMATION OFFICE: SUPPORTING THE RESEARCH COMMUNITY BY COLLECTING, ORGANIZING AND DISSEMINATING FOOD SAFETY RESEARCH INFORMATION

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The USDA Food Safety Research Information Office (FSRIO) at the National Agricultural Library (NAL) is a United States (U.S.) congressionally mandated program created to support the needs of the food safety research community. FSRIO meets its congressional mandate by collecting, organizing, and disseminating food safety research information through its Web site (http://fsrio.nal.usda.gov) and key information products which include the Food Safety Research Projects Database and the Automated Peer-Reviewed Journal Publication Feeds. The Research Projects Database (http://fsrio.nal.usda.gov/nal_web/fsrio/advsearch.php) is publicly accessible and showcases food safety research projects funded by both United States and International government agencies, as well as other private or non-government organizations. The information provided by this database can assist in the assessment of food safety research trends, identification of research gaps, and avoidance of unnecessary duplication. Currently the Database provides access to more than 8,000 food safety research projects and is the largest searchable collection of food safety research conducted among U.S. and International government agencies. The Automated Food Safety Peer-Reviewed Journal Feeds (http://fsrio.nal.usda.gov/research-projects-and-publications/research-publications-peer-reviewed-journals) provide ready access to the latest food safety research publications, including ahead of print. The publications can also be filtered by a specific journal or pathogen/contaminant of interest. FSRIO's publicly available website and information tools provide unique access to the latest food safety research and is a valuable tool for researchers and policymakers. The FSRIO is a collaborative project with USDA's Agricultural Research Service National Program 108: Food Safety and also works cooperatively with the University of Maryland's Nutrition and Food Science Department.

Keywords: *Food safety research information, research projects database, publications, FSRIO*

H-65
NOSHAN: SAFE AND FUNCTIONAL FEED FROM FOOD WASTE

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Food processing activities in Europe produce large amounts of by-products and waste. Roughly one third of the food produced in the world for human consumption every year (approximately 1.3 billion Tons) gets lost or wasted, according to a FAO-commissioned study. The food loss and waste generated per capita in Europe and North America is 280–300 Kg/year and between 95–115 kg/year, respectively, while consumers in sub-Saharan Africa and South and Southeast Asia only waste around 6–11 kg/year. Among the type of food, fruits (16.4%) and vegetables (25.8%), have the highest wastage rates of any food according to data from UK. Such waste streams are only partially valorized at different value-added levels (such as spreading on land, animal feed or composting), whereas the main volumes are managed as waste of environmental concern. Food loss and waste also amount to a major squandering of resources, including water, land, energy, labor and capital and needlessly produce greenhouse gas emissions, contributing to global warming and climate change The main focus of NOSHAN is to address the process and technologies needed to use food waste for feed and feed additives production at low cost, low energy consumption with maximal valorisation of starting wastes materials. Nutritional value and functionality according to animal needs as well as safety and quality issues are investigated and addressed as main leading factors for the feed production using food derived waste (fruit/vegetable/plant and dairy). NOSHAN unites 12 partners from different European countries (Belgium, France, Germany, Italy, Netherlands, Spain and Turkey) representing different segments of the R&D environment (industry leaders, SMEs, technological research centres and universities). Based on their broad experience, the consortium will investigate innovative and eco-efficient food waste conversion technologies to produce bulk feed components and feed additives.

Keywords: *Food waste, feed, low cost, low energy consumpiton*

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H-66
PRESSURISED HOT WATER EXTRACTION - GOOD TOOL FOR ISOLATION OF ALKALOIDS FROM SPICES AND HERBAL SAMPLES

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The pressurised hot water extraction (PHWE) belongs to the one of the fields called „green chemistry" and its major advantage is the reduction of organic solvents consumption. PHWE uses water as an extraction medium. Water is non-flammable, non-toxic, readily available, cheap and environmentally friendly solvent that could easily replace conventional organic solvents in extraction process. The change in its physicochemical properties at elevated temperatures and pressures enhances its usefulness. Attention is focused mainly on the use of unique devices for PHWE (working temperature range up to 280°C). Extraction conditions should be optimized for the extraction of different types of analytes from various matrices showing the highest efficiency. Optimised parameters are, in particular, extraction temperature and time, and the addition of modifiers and/or additives. Extracts are analysed by common separation methods such as using HPLC/UV/MS, GC/MS or CE depending on the type of target analytes. Evaluation of the extraction efficiencies of PHWE is applied on different compounds from a variety of sample matrices like food samples, plants, and environmental soils/sediments. The PHWE is also used in sample preparation to extract organic contaminants from foodstuff for food safety analysis and soils/sediments for environmental monitoring purposes. It is noted that there is a steady growing trend to use PHWE to extract bioactive and nutritional compounds from food and plant materials. In this study the modern extraction methods PHWE has been optimised and used for the isolation of main alkaloids from spices and herbal samples. In this case capsaicin and dihydrocapsaicin were monitored in chilli peppers while nicotine, nornicotine, anabasine and 4,4'-bipyridyl were determined in the tobacco samples. This optimisation has been performed according to the central composite design approach followed by the evaluation of individual conditions using the surface response modeling.

Keywords: *Pressurised hot water extraction (PHWE), Alkaloids, Chilli peppers, Tobacco*

H-67
QUICK SUPRAMOLECULAR SOLVENT-BASED MICROEXTRACTION FOR QUANTIFICATION OF LOW CURCUMINOID CONTENT IN FOOD

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There is a need to monitor the consumption of curcuminoids, an EU-permitted natural colour in food, to ensure the acceptable daily intakes are not exceeded, especially by young children. A sensitive method able to quantify low contents of curcumin (CUR), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) in foodstuffs was here described. It was based on a single-step extraction with a supramolecular solvent (SUPRAS) made up of reverse aggregates of decanoic acid and direct analysis of the extract by liquid chromatography-photodiode array (PDA) detection. The extraction involved the stirring of 200 mg of foodstuff with 600 µL of SUPRAS for 15 min. No cleanup or concentration of the extracts was required. Driving forces for curcuminoid solubilisation were dispersion and hydrogen bond. The method was applied to the determination of curcuminoids in different types of foodstuffs (i.e. snack, gelatine, yogurt, mayonnaise, butter, candy and fish products) tha t encompassed a wide range of protein, fat, carbohydrate, sugar and water content (i.e. 0.85–11.04, 0–81.11, 0.06–75, 0.06–79.48 and 10.08-85.10 g/100 g of food, respectively). Method quantification limits for the foodstuffs analyzed were in the ranges 2.9–7.7, 2.8–11.2 and 3.3–9.0 µg kg^{-1} for CUR, DMC and BDMC, respectively. The concentration of curcuminoids found in the foodstuffs and the recoveries obtained from fortified samples were in the ranges ND-284, ND-201 and ND–61.3 µg kg^{-1} and 82–106, 89–106 and 90–102% for CUR, DMC and BDMC, respectively. The relative standard deviations were from 2 to 7%. This method allowed the quick and simple microextraction of curcuminoids with minimal solvent consumption while delivering accurate and precise data.

Keywords: *Curcuminoids, supramolecular solvent, microextraction, food*

H-68
ANALYSIS OF SYNTHETIC DYES IN FOOD SAMPLES BY UHPLC–DAD

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Dyes are used as food additives to improve the appearance of food. Initially, natural dyes were used, but from the XIX century synthetic organic colorants are the most commonly utilized by the food industry. At present, in the European Union, only 14 synthetic colorants are permitted as food additives, and maximum residue levels, in the mg kg^{-1} range, have been established in a wide range of food products. The laboratories in charge of official controls, which have to analyze a large number of samples, need simple, high throughput and robust methods, suitable for a large variety of samples. Since synthetic colorants are polar compounds, liquid chromatography is the technique of choice. On the other hand, due to the spectral characteristics of these compounds, the detection at the visible zone of the spectra is an excellent option, because it is selective and sensitive enough, and moreover is very robust and simple. In addition, diode array detectors (DAD) allow for confirmatory analysis, being detection by mass spectrometry necessary very occasionally. Here we present a proposal of analytical schemes for the analysis of 14 synthetic colorants in a wide range of food samples. For candies and sweets extraction with water at 60°C and mechanical shaking provides good results, whereas pressurized liquid extraction with a water/ethanol/ammonia mixture is suitable for meat, fish, snacks and related products. The extracts from snacks, candies, sweets and canned vegetables are adjusted to pH below 4 and cleaned-up by solid phase extraction with polyamide cartridges. On the other hand, liquid-liquid extraction with hexane is used for the clean-up of meat and fish extracts. The purified extracts are injected in the chromatographic system. The separation is performed in ultra-high performance liquid chromatographic (UHPLC) conditions with a C18 column and a gradient elution based on acetonitrile:methanol:aqueous ammonium acetate mixtures. A chromatographic run takes 12 min. The whole spectrum is acquired for confirmation purposes, but for quantification four wavelengths (450, 490, 520 and 620 nm) are used. The methods have been successfully validated in several food matrices, grouped into five categories: soft drinks, candies and sweets, canned vegetables, meat and fish products, and cereal based products.

Keywords: *Synthetic dyes, extraction, clean-up, liquid chromatography*

H-69
HIGHLIGHTS IN 1H NMR SPECTROSCOPY OF WINE – MODEL STUDY ON GERMAN WINE

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Nuclear magnetic resonance (NMR) spectroscopy is nowadays of growing importance in quantitative and qualitative analysis of wine, named targeted and non-targeted wine analysis. In this study on German wines (n = 1383 from all 13 wine-growing areas in Germany) the authenticity, the grape variety, the geographical origin, and the year of vintage of wines were investigated by ^{1}H NMR spectroscopy and stable isotope analysis (SNIF–NMR, ^{18}O, ^{13}C) in combination with several steps of multivariate data analysis including Principal Component Analysis (PCA), Soft Independent Modelling of Class Analogy (SIMCA) and Linear Discrimination Analysis (LDA) and others. Different chemometric models constructed using sub-sets of the data (namely, when one or two of above-mentioned parameters are set constant) were developed and validated using test data set. Separate models for classification of five red wine grape varieties (Spätburgunder, Dornfelder, Regent, Lemberger, Sch warzriesling) and five white wine grape varieties Grau/Weissburgunder, Kerner, Riesling, Müller Thurgau, Silvaner) have been built. On the basis of the statistical evaluation of NMR spectra, the decision tree for chemometric evaluation of three parameters with and without a priori knowledge about a wine sample has been proposed. In combination of ^{1}H NMR data with stable isotope data the correct prediction of origin could be increase up to 100% whereas stable isotope data resulted only in 60–70% correct prediction and NMR data in 82–89% respectively. Moreover, high-field ^{1}H NMR spectra of wines provide ideal conditions for targeted analysis, which means the unambiguous identification and subsequent quantification of a number of individual compounds.

Keywords: *NMR spectroscopy, German wine, chemometrics, stable isotope*

H-70
FABRICATION OF FUNCTIONALIZED MAGNETIC NANOPARTICLES FOR THE DETERMINATION OF ENDOCRINE DISRUPTORS IN MILK SAMPLES

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EDCs are chemicals that interfere with endocrine (or hormone system) and are of an increasing concern because of their potential impacts on the environment, wildlife and human health. The European Commission has published a draft list of chemicals, which are believed to damage health by interfering with the way hormones work. The range of substances reported to cause endocrine disruption is diverse including certain organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), selected because of their potential for human exposure and their high production volumes. Food is likely to be one of the most important routes of human exposure to EDCs. In the present study, a novel magnetic solid phase microextraction (MSPE) based on C18-functionalized magnetic silica nanoparticles (C18-NPs) as sorbents was developed for the determination of endocrine disruptors (EDCs) – 20 organochlorine pesticides and 6 PCB congeners – in milk samples by gas chromatography- mass spectometry (GC-MS). The resultant magnetic nanoparticles were characterized by several spectroscopic techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform-infrared (FTIR). The magnetic solid phase extraction (MSPE) was performed by dispersion of the Fe_3O_4-SiO_2-C_{18} nanoparticles in 3 mL of milk samples with sonication, after protein precipitation. Then, the sorbent was collected by applying of an external magnetic field and the analytes was desorbed by appropriate solvent system. Various parameters (washing and elution solvents, amount of sorbents, time of extraction and elution) affecting the adsorption of EDCs by the C18-NPs composites have been investigated and optimized by means of experimental design and response surface methodology (RSM). Under the optimum conditions, average recoveries ranged from 73% to 104%, the calculated calibration curves gave high-level linearity for all target analytes with correlation coefficients above 0.9972 and the obtained limits of detection (LODs) and quantification (LOQs) were in the in the low µg/L level. The proposed method was used to analyze milk samples of Epirus region (NW Greece) selected from local markets.

Keywords: *Magnetic solid phase extraction (MSPE), functionalized magnetic nanoparticles, EDCs, milk, GC-MS*

Acknowledgement: *This research project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY- MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013)*

H-71
VALIDATION OF A ROUTINE NOROVIRUS REAL-TIME REVERSE TRANSCRIPTASE PCR ANALYSES IN FOOD SAMPLES

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Already for a long time Norovirus is known as case for outbreaks of acute gastro-enteritis. In late 2012 more than 11,000 children across Germany suffered from an uncomfortable gastrointestinal illness triggered by Norovirus contaminated school and nursery school lunches. The Center for Disease Control and Prevention reported 145 confirmed outbreaks in the USA only for 2011. The outbreak of this illness strikes all categories of the population and it more than 90% of all cases the genotype II of the Norovirus is responsible. People are known to be the only reservoir of human Norovirus infections and the infection takes place via 3 main ways: food infections, from person to person and via water. For food shellfish is a common vector. As shellfish is filtering a huge amount of seawater, which can be contaminated with fecal waste, the tissue of shellfish and crustacean are often accumulated with high levels of pathogens. Consumption of raw or insufficient cooked crustacean and shellfish is an important risk factor for outbreaks in foodborne infections, including Norovirus. Although the control of several food agencies is focused on shellfish and crustacean also vegetables, red soft fruit and water can be infected. The goal of this study was to validate a commercial routine application for the qualitative detection of Norovirus in food samples. The validated method should be fit for accreditation based on the ISO 17025 standard. As it is difficult to cultivate and quantify viruses by classic (microbiological) techniques and there are no specific culture media to identify Norovirus, the method of choice to identify Norovirus in food samples was a commercial real-time PCR kit. As main food matrices for the validation we chose shellfish and crustacean, fruit and vegetables, and water. The general principle of the virus detection test is based on 2 main steps. First the extraction of the virus, with focus on the extraction sufficiency and possible inhibition by using an internal virus control and secondly the actual detection using PCR. In all cases RNA is extracted from the obtained virus and amplified using reverse transcriptase (RT) as a first step followed by the amplification and detection of DNA using real-time PCR as a second step. The validation was built up in two steps:
- Extraction: proving the reproducibility of the virus extraction.
- Detection: proving specificity, repeatability, reproducibility and LOD of the real-time RT PCR.
Critical points which will be discussed are the lack of stable reference materials, the dissection of the mollusk samples, controlling the circumstances were under the virus extraction is done thus reducing inhibition and increasing extraction sufficiency and controlling the environment and time frame because of the instability of the extracted RNA. The validation was verified by participation in a proficiency test and accreditation audit.

Keywords: *Norovirus, PCR, Food Analytic, Validation*

H-72
INCREASING EXTRACTION EFFICIENCY OF WET SAMPLES USING A NOVEL NEW POLYMER DURING ACCELERATED SOLVENT EXTRACTION

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Accelerated solvent extraction is a high-temperature and high-pressure extraction technique that is widely used for sample extractions in the environmental, chemical and food analysis industries. Extractions at higher temperatures and pressures allow faster extraction of analytes relative to conventional solid-liquid based extraction techniques such as Soxhlet. Typically the sample is mixed with a dispersant and loaded into a cell followed by extraction with a suitable solvent. Analyte recovery using this method of extraction for wet samples is always challenging, as the presence of water in the sample can interfere with the extraction efficiency. The analyte of interest may partition between the extracting solvent and the water phase. It is therefore desirable to dry the sample prior to extraction. Traditional drying techniques that involve mixing the wet sample with an inorganic salt that has a high affinity for the aqueous phase are unsuitable for in-cell extraction. Additionally off-line drying methods like freeze drying are extremely tedious and time consuming. This study presents the use of a novel new polymer for in-line drying of a wet sample for the analysis of organochlorine pesticides, and polyaromatic hydrocarbons (PAHs) in different matrices. Data showing recoveries for each of these target compounds in different matrices will be presented.

Keywords: *Accelerated Solvent Extraction, Sample Preparation, Soxhlet, PAH; In-Line drying;*

Acknowledgement: *Texas Tech University, Lubbock, TX, USA, for sharing the experimental data on organochlorine pesticide analysis.*

H-73
FAST GC COMBINED WITH A HIGH-SPEED TRIPLE QUADRUPOLE MASS SPECTROMETER FOR THE SIMULTANEOUS ANALYSIS OF UNKNOWN AND TARGET CITRUS ESSENTIAL OIL ANALYTES

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The present research description is focused on the evaluation of a high-speed triple quadrupole mass spectrometer (QqQ MS), carried out under moderately-fast GC conditions. The MS device is capable of operation under high-speed GC conditions, in both full-scan (maximum scan speed: 20,000 amu/s) and multiple reaction monitoring (MRM) modalities. Furthermore, the QqQ system can generate full scan and MRM data at the same time, also in a very rapid manner. A fast method was developed for the: (i) qualitative analysis of untargeted essential oil constitents, and (ii) the quali/quantitative analysis of targeted ones, namely three preservatives (o-phenylphenol, butylated hydroxytoluene, butylated hydroxyanisole). The MS system generated a more-than-sufficient number of data points/peak for both identification and quantification purposes. The level of sensitivity, reached through the MRM mode widely exceeded the requirements of current legislation. Method validation, related to the targeted analysis, was also performed.

Keywords: *Fast gas chromatography, triple quadrupole mass spectrometry, food analysis, Citrus essential oil*

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H-74
THE EFFECTS OF THE COLLAGEN-CONTAINING KOREAN FOOD ON BONE METABOLISM

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As our communities enter into aging society, inclination to look young and healthy is spreading rapidly. As a response to this movement, large increases in research and consumption of collagen-containing foods that support skin care and joint-bone conditions are visible in and around East Asia. Among traditional Korean foods, there are those that contain large quantities of collagen such as Samgyetang (ginseng chicken soup), Eogeultang (soup made from Pollack skin), Doganitang (soup made from soft cow bones) and more. We investigated the effects of these collagen-containing Korean foods on bone metabolism from cellular biological perspective and analyzed the foods' various functionalities. Bone health is maintained through a balance between osteoblast that forms new bones and osteoclast that handles bone resorption of aging bones. Evaluation of bone metabolism was performed by detecting the main cellular factor, Tartrate-Resistant Acid phosphate (TRAP), in osteoclastogenesis, and representative cellular protein, alkaline phosphatase (ALP), in osteoblast differentiation. Hence, the activity of osteoclastogenesis was measured using TRAP staining assay while ALP assay was used for measuring the differentiation activity of osteoblast. According to this research using the ethanol extracts from the foods, one of the collagen-containing foods, Samgyetang, stimulated the activity of osteoblast differentiation. On the other hand, Eogeultang was found to be effective in limiting the osteoclastoge nesis activity. Supposing that osteoporosis is an outcome of decrease in osteoblast cell number from aging and destruction of bone balance from increase in osteoclast cell number, Eogeultang can be effective in subduing this bone disease. Samgyetang can be potent also, as it profoundly catalyzed the osteoblast differentiation activity according to this research. In particular, 'Advanced Samgyetang' – a novel development where supplementary materials from traditional Korean food culture were added to the original recipe – showed about 60% increase in its catalyzing power. Also, 'Advanced Eogeultang', with its enhanced collagen content from ingredient control, showed about 30% increase in its capacity to limit the osteoclastogenesis activity. Through our cellular biological experiment, it was observed that collagen content in food is capable of affecting healthy bone metabolism.

Keywords: *Collagen, collagen containing Korean food, bone metabolism*

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H-75
VALIDATION OF AN ALTERNATIVE METHOD FOR SALMONELLA DETECTION IN FEED: A PRAGMATIC APPROACH TO THE CHALLENGES

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Many novel methods for pathogen detection in food and feed have been published in the scientific literature, but few have been subsequently employed in actual analytical practice. A major reason for this lack of implementation is that the development of these methods has not been taken forward to internationally validation. A standard procedure exists (ISO 16140) which describes the steps required to validate an alternative method against a reference method; the procedure is intensive and logistically challenging, especially when the refernce method is compex, and also when infectious materials need to be used in concert by each partner. A loop-mediated amplification-based method for detection of Salmonella in soya meal is currently being taken through a validation procedure. The challenges encountered are described, along with the approaches to address them.

Keywords: *Salmonella, detection, alternative method, validation*

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H-76
A LOOP- MEDIATED AMPLIFICATION-BASED DETECTION METHOD FOR SALMONELLA IN SOYA MEAL

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Loop-mediated amplification (LAMP) is an alternative nucleic acid amplification to the polymerase chain reaction (PCR). A LAMP-based method has been developed for the detection of Salmonella in soya meal. The method is based on the assay of Zhang et al. 2011 (Appl. Env. Microbiol. 77 6495-6501), modified to contain an internal amplification control to identify correctly performed reactions. The assay is harnessed to a sample treatment comprising standard Salmonella culturing procedures, to be fully compatible with ISO 6579. The method should be useful for analysis of a wide range of feedstuff materials for the presence of Salmonella species.

Keywords: *Loop-mediated amplification; Salmonella; detection; control*

Acknowledgement: *The research leading to to these results has recieved funding from the European Union Seventh Framework Programme (FP7 2007-2013) under Grant Agreement No 265702.*

H-77
EXPLORING RANCIDITY ASSESSMENT AND PREDICTION USING THE OXITEST

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Rancidity is caused by lipid oxidation and has a major impact on the product's shelf-life, negatively affecting taste, smell, texture and nutritional quality. Being able to assess rancidity is an important part of a product's development and control. There are a number of different analytical tests available to assess rancidity or a food's potential for oxidation. Measurement of peroxide value, anisidine value and free fatty acids are used to determine the extent of lipid oxidation which has occurred in the product, and can be used to compare samples taken at different times over the shelf life. These measurements can also be used in conjunction with accelerated shelf life trials, where samples are stored at higher temperatures in order to accelerate ageing of the product. A potential problem with these methods is that fat must be extracted from the product before analysis, which could itself alter the degree of oxidation of the sample. The measurements described above are made on aged products, but there are circumstances where an indication of the oxidative potential may provide the required information, for example in the comparison of different oils or for an initial assessment of the effect of an antioxidant. Measurement of the oxidative potential of an oil or fat can be carried out using an instrument such as the Rancimat, which is well known and has been used for many years. However, as for the analytical measures described above, for most products the oil or fat must be extracted first which may not only have an impact on the degree of oxidation of the sample but removes the fat from any other components within the product that may affect lipid oxidation. To overcome this issue, assessment of the oxidation of the whole product is offered by a relatively new instrument – the OXITEST. This instrument has been designed to analyse lipid oxidative stability on whole products without the need for fat extr action, which could offer a major advantage when assessing particular products. This project was designed to use the OXITEST alongside established techniques to assess lipid oxidation in fried potato crisps. Potato crisps were fried using two different oils, with one oil containing an antioxidant and the other without. The crisps are currently being stored under two different temperature conditions, 20 and 30°C. At selected time-points samples will be removed from storage and analysed using a variety of rancidity assessment methods. Rancidity measures will be taken over the shelf life of the product stored under ambient and accelerated conditions. The final set of data will be used to compare the predictive abilities of Rancimat and Oxitest data with respect to oils and finished product.

Keywords: *Rancidity, crisps, oxidation, shelf life*

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H-78
COMBINATION OF MALDI–MS, DART–MS AND 1H-NMR SPECTROSCOPY IN THE STUDY OF EDIBLE OILS COMPOSITION

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Edible oils are complex mixtures that contain mainly triglycerides (TAG; their content in oils is of 95-98%) and the non-saponifiable fraction (phytosterols, tocopherols, hydrocarbons, squalene, fatty alcohols, and some other components). The composition of fatty acids in dietary fats and oils plays the important role in human nutrition. Excessive saturated fats are considered to cause the unfavorable health effects, while cis-isomers of mono- and polyunsaturated fatty acids are assumed to effect positively on human health. So, characterization of edible oils composition is necessary for food quality control. The most frequently used techniques for vegetable oils analysis are gas and liquid chromatography with different detection systems. The clue point of these techniques application is the time-consuming sample preparation. Unfortunately, every step of this process can change a sample composition and cause mistakes in results of analysis. In the present study an alternative approach for direct determination of the edible oils composition without preliminary derivatization and chromatographic separation on the basis of MALDI and DART mass spectrometry and quantitative 1H-NMR spectroscopy has been developed. The approach was tested on more than 25 different types of oils. It was shown, that both MALDI and DART mass spectra can be used as "fingerprints" and provide information about the TAG composition in edible oils. At the same time, MALDI mass spectra provide no information concerning minor components of the oil (the non-saponifiable fraction) due to their low ionization efficiencies. DART mass spectra, however, reveal the ion peaks of these components, so this method is better for characterization of complete oils composition. 1H-NMR spectroscopy data allows to determine complex parameters of the oils: content of saturated and unsaturated acids, hydroxyl and iodine values etc.

Keywords: *edible oil, MALDI-MS, DART-MS, 1H-NMR spectroscopy*

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H-79
GENERALISED FUZZY HOUGH TRANSFORM: AN ALIGNMENT TOOL FOR COMPLEX DATA

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Complex data can be generated e.g. by non-targeted analysis of food samples. The data is complex partly because of peak shifts between samples. Warping is an alignment tool that has widespread use in chromatography to correct peak shifts. However, in complex datasets nearby peaks can shift differently, and even change elution order. In such situations warping will fail. In NMR peaks change order regularly and therefore require different alignment methods. The Generalised Fuzzy Hough Transform (GFHT) is an alignment algorithm that can deal with nearby peaks shifting in different directions and changes in peak order [1; 2]. Here we have adapted GFHT for chromatography [3]. The principle behind GFHT is that peaks shift depending on the same underlying causes, such as temperature or pH. There is no need to identify the underlying cause, rather the peak shifts can be modelled from shifts from other peaks, or the components of a principal component analysis of the shifts of other peaks. Using the fact that peak shifts are predictable, we can correctly align peaks in difficult situations where there are ambiguities about the peak identity. One example is where most samples have one peak but some have two; which of the two peaks belong to the one of the majority of samples? The GFHT algorithm exploits the multisample advantage to solve this ambiguity with ease. The multisample advantage has largely been overlooked in processing of chromatographic data. We expect to see an increase in the number of algorithms using this multisample advantage for various parts of data processing.

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Keywords: *metabolomics, liquid chromatography, mass spectrometry, alignment, warping*

GENETICALLY MODIFIED ORGANISMS (GMO´s)

(I-1 – I-7)

I-1
VALIDATIONS OF THE GMO ANALYSIS METHODS IN THE ACCREDITED LABORATORY

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In European Union laboratories that are responsible for official control of genetically modified food or feed must be accredited according to the ISO/IEC 17025 standard "General requirements for the competence of testing and calibration laboratories". Management Requirements and Technical Requirements are the most important parts of ISO/IEC 17025. Technical requirements that are primarily related to the tests performed in the laboratory includes all factors which determine required correctness and reliability of the applied method. Accredited laboratory has to fulfill standard requirements to ensure quality of their results. Moreover accreditation is a good way to prove laboratory's technical competence and recognize test results by various parties. All analytical methods used in accredited laboratory have to be validated. Validation procedure should compromise technical and financial conditions and describes theoretical criteria for the performance of analytical method. The validation process consists of setting several parameters, like: accuracy, limit of detection, selectivity, linearity, repeatability, reproducibility, uncertainty and verifying if the particular methods meets specified criteria. National Reference Laboratories for GMO analysis have to adapt ISO/IEC 17025 requirements to GMO testing. The European Union Reference Laboratory for Genetically Modified Food and Feed and European Network of GMO Laboratories support GMO testing laboratories by determining important parameters for validation of qualitative and quantitative methods. There are many methods for detection and quantification of GMO but each of them should be validated and verified before use. The aim of this work is to present how small laboratory that analyze GMO can fulfill ISO/IEC 17025 standard requirements.

Keywords: *Validations of the GMO analysis methods, ISO/IEC 17025 standard, accredited laboratory*

I-2
NEW APPROACH OF A QUALITATIVE METHOD VALIDATION APPLIED TO THE DETECTION OF ROUNDUP READY® SOY IN SOYBEANS BY NESTED PCR

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Considering the expansion of genetically modified soybean and the basic principle of consumers' right to access information about the products, the Brazilian and other countries legislation establishes a limit of genetically modified organisms (GMO) to label transgenic food. Qualitative tests based on polymerase chain reaction (PCR) have been recommended for the GMO analysis in food – such as Nested PCR. However, the validation of qualitative methods, important to confirm the reliability of analytical results, is still a critical point in the quality management of food analysis laboratories. The main of this work was to validate an in-house method for qualitative detection of Roundup Ready (RR®) soy in soybeans by Nested PCR. Certified Reference Materials (CRM) of genetically modified RR® soy and blank samples or 100% conventional soy (provided by a certified producer) were used to obtain formulations at nine concentration levels (0,001; 0,005; 0,010; 0,015; 0,020; 0,025; 0,030; 0,035 e 1,000% of RR® soy). For the selectivity evaluation, formulations were prepared with CRM of Bt11 and GA21 maize in the presence and absence of RR® soy. Techniques for quantification of the deoxyribonucleic acid (DNA), agarose gel electrophoresis and fluorimetry, were compared. The validation procedure, proposed by GONDIM (2012), was designed for the evaluation of rates (false results, sensitivity, selectivity and reliability), accordance, concordance, limit of detection, unreliability region and robustness. The results indicated false-positive rate of 0% and selectivity and reliability rates of 100.0% for both techniques, demonstrating selectivity of the method. Sensitivity and reliability rates ranged from 23.3 to 100.0% for agarose gel electrophoresis and from 30.0 to 100.0% for fluorimetry. For all levels above 0.030% of RR® soy were obtained 100.0% of positive results, confirming the sensitivity of the method. The unreliability regions were estimated between 0 and 0.007% of RR® soy and between 0 and 0.005% of RR® soy for agarose gel electrophoresis and fluorimetry, respectively. Then, estimated detection limits, corresponding to 95% of positive results, were 0.007% for the agarose gel electrophoresis and 0.005% for fluorimetry. The method was considered selective for maize events Bt11 and GA21 and robust for different concentrations of target DNA and brands of Taq DNA polymerase. A paired t test was applied to compare the techniques for quantification of DNA, indicating best performance of the fluorimetry ($p < 0.05$). The applicability of the method was demonstrated by analysis of commercial samples collected by the Health Surveillance of Minas Gerais State, Brazil, in 2012. The validated method was fit for the purpose, considering the maximum limits to label GMO in food established by the Brazilian and international legislation.

Keywords: *In-house validation, qualitative assay, Roundup Ready soy, genetically modified organisms, Nested PCR*

Acknowledgement: *CAPES, FAPEMIG, FUNED*

I-3
DEVELOPMENTS IN GMO TESTING: A NEW SEMI-QUANTITATIVE LATERAL FLOW COMB USING A PORTABLE LATERAL FLOW DEVICE READER

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For the presence of GMO traits in grains Lateral Flow Devices for rapid, qualitative testing are often the method of choice. These rapid tests are widely used in the field for screening purposes. Meeting the market demands for quantification of test results and being able to test for more straits at once, a comb format was developed which can be used as semi-quantitative test method. For the test a new strip reader, the AgravisionTM reader is used to analyze the results rather than just relying on a visual reading, as is common practice in the qualitative methods. GMO tests are Lateral Flow Devices and are based on a sandwich ELISA format. Samples are extracted with a buffer, and the extracts are allowed to flow up the device. The incubation time is only five minutes and afterwards presence or absence of a visual marker on the device indicates the presence or absence of a selected GMO trait in the sample. This marker varies in the intensity with varied concentrations of the analyte in the sample. This variation in intensity of the visual marker, combined with the AgraVisionTM Reader measuring the reflectance or optical density of the line, is used to semi-quantitatively measure the trait in a sample. Method validation studies were performed for the GMO Comb, which include tests for CP4 EPSPS, Bt-Cry1Ab, Bt-Cry3Bb1, Bt-Cry1F, Bt-Cry34Ab1, PAT, and VIP3A. These methods are semi-quantitative over a range of 0.5–5% corn modified seed in unmodified seed when the test is performed at room temperature. The limit of detection is 0.5% or lower for above listed traits in corn. The AgravisionTM reader uses digital imaging software and therefor can be used for this semi-quantitative method. Further, results are independent of the individual user's visual readings. This poster will present the development of a semi-quantitative method for testing GMO traits with a comb using Lateral Flow devices. Data on accuracy and precision will be demonstrated as also results of the study.

Keywords: *GMO Testing, Lateral Flow Comb, Semi-quantitative test, Portable Lateral Flow Device Reader*

I-4
DEVELOPMENT OF A NOVEL STANDARD PLASMID FOR DETECTING 3 EVENTS OF UNAPPROVED GENETICALLY MODIFIED PAPAYA

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Unapproved genetically modified (GM) papaya events with various traits have been developed and there has been a need for monitoring them. GM papaya has not yet been approved for food in Korea. In this study, a novel standard plasmid (pGEM-PAPAYA3) was constructed for qualitative and quantitative detection of unapproved GM papaya, 55-1, 16-0-1 & 17-0-5, and Huanong No.1. The papain gene was used as an endogenous gene for detecting 3 kinds of unapproved GM papaya. Event-specific primers and probes were designed based on the genomic flanking sequences of 55-1, 16-0-1 & 17-0-5, and Huanong No.1. Each target was cloned into the pGEM vector. Before using as a positive control and a real time PCR standard, the plasmid DNA was digested with a restriction enzyme SpeI and was purified with the QIAquick gel extraction kit to reduce genomic DNA contamination. Qualitative and quantitative PCR assays with the pGEM-PAPAYA3 were established employing the designed event-spec ific primers and probes. The limit of detection of qualitative PCR assays was approximately 1 to 100 copies. Furthermore, the quantitative PCR assays for detection of 3 kinds of unapproved GM papaya were validated in-house by two different researchers. Five dilutions of reference molecule (corresponding to 20, 200, 2000, 20000, and 200000 copies each reaction) were used to establish calibration curves for 55-1, 16-0-1 & 17-0-5, and Huanong No. 1 The square regression coefficients (R^2) were 0.993–0.997 for each target. These results indicate that the standard reference plasmid is applicable for detecting 3 kinds of unapproved GM papaya as a convenient positive control and a reliable calibrator.

Keywords: *Genetically modified papaya, novel standard plasmid, qualitative, quantitative, PCR*

I-5
DEVELOPMENT OF EVENT-SPECIFIC MULTIPLEX PCR METHOD FOR FOUR EVENTS OF GM CANOLA

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Different events of genetically modified (GM) canola have been developed to have herbicide tolerance for human food (oil) and livestock feed consumption. In this study, event-specific detection method using multiplex PCR was developed to identity 4 kinds of GM canola, T45, GT73, Rf3 and Ms8. These events of GM canola have been approved for food and feed in Korea. The fatty acyl-ACP thioesterase (FatA) gene was used as an internal control for detecting 4 kinds of GM canola. Event-specific primers were designed based on the 3' and 5'-flanking region of 4 kinds of GM canola. Qualitative multiplex PCR assay was established employing the designed event-specific primers and PCR products of the expected sizes were confirmed by agarose gel electrophoresis and sequencing. We also monitored canola seed samples collected in food and feed companies in Korea using this PCR method. Consequently, GM canola GT73, Ms8, and Rf3 were found in seed samples imported from Canada and China. The results indicate that the methods can be efficiently used for detecting four GM canola events.

Keywords: *Genetically modified canola, event-specific, detection method, multiplex PCR*

I-6
EVALUATION OF STABILITY OF PRE-SPOTTED AND READY-TO-USE PLATE FOR GMO DETECTION TO ASSIST A NATIONAL BIOSAFETY PROGRAM

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The labeling of food products containing genetically modified organisms (GMO) is mandatory in many countries, including Brazil, to ensure the access to information for helping the consumer choice. Given the commercial releases and labeling regulation, the challenge is to develop methods for GMO detection that may accompany the increasing number of new events released on the world market. The constant increase of different GMOs shows the need to develop multi-target methods. In this way, the objective of this work was to evaluate the stability of a fast and ready-to-use system for different GM events detection using pre-spotted plates by lyophilized reagents, including TaqMan™ probes, for real time PCR runs. The pre-spotted lyophilized plates were stored at -20°C until analyses (7 14, 30, 60 and 365 days), when the reagents were re-suspended to PCR reaction ran in a SDS ABI PRISM 7000 (Applied Biosystems). The results showed that the GM events (RR and MON89 788 soybean; MON 863, MIR604 and DAS 59122-7 maize), were detected in pre-spotted plates, presenting similar reaction efficiency (83% to 105%) when compared to fresh plates, without the lyophilized reagents. In addition, the comparison between Threshold Cycle (Ct) of each reaction curve from pre-spotted and fresh plates showed minimal loss in the reaction yielding in all tested storage time. These results showed that pre-spotted lyophilized plates is suitable for multi-GMO detection, supporting any National Biosafety Program for monitoring GMOs.

Keywords: *GMO Detection, q-PCR, Ready to Use plates*

Acknowledgement: *Embrapa and National Council for Scientific and Technological Development - CNPq*

I-7
MONITORING OF ILLEGAL USE OF SOYBEAN IN PROCESSED TURKISH MEAT PRODUCTS AND DETECTION OF GENETICALLY MODIFIED SOYA

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In Turkey the use of soybean is not permitted for use in meat products as a low-cost meat replacement even if appropriately labelled (Regulation No: 2012/74).In order to screen for possible illegal use of soybean the performance characteristics of a commecial PCR kit for detection of soybean DNA in raw and cooked meat products was established. Comminuted chicken and beef products containing soybean levels from 0.1–10.0% were prepared and analysed by real-time PCR to amplify the soybean lectin gene. For both raw and meatscooked for 20 min at 200°C, the PCR method could reliably detect the addition of soybean at a level of 0.1%. A survey of 38 Turkish processed meat products such as soudjouk, meatball, Turkish ravioli and doner kebap was conducted. 6 samples were negative for the presence of soybean, but 32 were found to be positive. Of the soybean positive samples further DNA analysis was conducted by PCR to amplify any recombinant DNA to detect whether g enetically modified ('Roundup Ready') soybean had been used. Positive samples for the presence of 2 soybean were subjectedto a real-time quantification of GMO using a duplex real-time PCR. The results showed that out of 38 samples 2 were positive for 'Roundup Ready' soybean.

Keywords: *PCR, soybean, fraud, gmo, meatproducts*

Acknowledgement: *R-Biopharm Rhône Ltd for kits*

MYCOTOXINS, MARINE AND PLANT TOXINS

(J-1 – J-71)

J-1
NEW ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY–HIGH RESOLUTION TANDEM MASS SPECTROMETRIC METHOD FOR QUANTIFICATION OF 323 PESTICIDES, 55 MYCOTOXINS AND 11 PYRROLIZIDINE ALKALOIDS

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Currently, development of fast and reliable analytical methods for analysis of a wide range of potential industrial or natural contaminants is highly desirable for control of various agriculture products. Major compounds of interest in this area are performed by pesticides, chemicals meant for preventing of crops from pests, mycotoxins, toxic secondary metabolites of microscopic filamentous fungi, and also pyrrolizidine alkaloids, toxic secondary metabolites produced directly by certain plants. For the qualitative or quantitative analysis of the above mentioned substances, liquid chromatography with tandem mass spectrometric detection is being often used. However, nowadays, using of new analytical strategies exploiting tandem mass spectrometry performed with high-resolving power mass analyzers continues to become a great challenge. Usually a significant sensitivity, high mass resolution, and accurate mass measurement ability (HR/AM) are provided by these typ es of instruments. The goal of our study was to develop a rapid, simple, and high throughput method for separation and detection of large number of analytes (323 pesticides, 55 mycotoxins and 11 pyrrolizidine alkaloids are included). The ultra-high performance liquid chromatograph Dionex 3000 RSLC coupled with quadrupole-orbitrap high resolution tandem mass spectrometer Q-ExactiveTM (Thermo Fisher Scientific) were used in this particular case. For sample preparation, optimized quick, easy, cheap, effective, rugged and safe (QuEChERS) strategy was used.

Keywords: *Pesticides, mycotoxins, pyrrolizidine alkaloids, orbitrap, U–HPLC–HRMS/MS*

Acknowledgement: *Financial support from specific university research (MSMT No 20/2013).*

J-2
DETERMINATION OF AFLATOXIN LEVEL IN HAZELNUT GROWN IN AZERBAJIAN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Due to the favourable climatic conditions, there are opportunities to grow all kinds of plants including hazelnut in Azerbaijan. Hazelnut, which is a source of Vitamin E, Vitamin B, copper, protein, and folic acid, has arginine positive impact on blood pressure, anaemia diseases as well as in reduction of cholesterol. Hazelnuts are grown mostly in the north and north-west regions of Azerbaijan. In order to assess the controls on aflatoxin contamination in hazelnuts intended for export to the European Union (EU), Food and Veterinary Office made a follow up on audit and recommended that the control laboratories must comply with the ISO/IEC 17025. Therefore, in the scope of the present study, the daily routine analysis method was validated and some representative samples shipped to laboratory were analysed to provide the aflatoxin level in hazelnuts grown throughout Azerbaijan. The method used in routine analysis (Senyuva and Gilbert., 2005) is based on an immunoaffinity column clean-up and reversed-phase LC with post column derivation achieved with electrochemically generated bromine (Kobra Cell) followed by fluorescence detection. For validation studies, blank hazelnut paste were spiked at four levels of 0.6 ng/g, 1 ng/g, 2.5, and 7 ng/g with a mixture of aflatoxins B_1, B_2, G_1, G_2. Two technicians being familiar with the method performed the validation during several days. The work was distributed between them, thus, the precision data reflects reproducibility within laboratory. The experimental results revealed that the relative standard deviation for repeatability (RSD_r) and for reproducibility (RSD_R) is within the criteria stated in (EC) No 401/2006. Moreover for monitoring the validity of the method, the laboratory participated into the proficiency test organized by a well-known provider and the results are within the acceptable Z score. Then, representative samples taken from 11 different lots of hazelnut grown in Sheki, Zagatala and Gakh regions were analysed in 2012 and 2013. It has observed that aflatoxin B_1 level changed between 0.1 ppb to 2.3 ppb, which is not above the maximum residue level set as 5 ppb both in EU and national standards. Total aflatoxin level also remained within the permitted level. The study is still under progress, and the aflatoxin level in hazelnut samples grown in different region of Azerbaijan will be continued to be monitored either for domestic consumption or for its intended export to EU countries.

[1] Şenyuva, H. Z. and Gilbert, J., 2005, Journal of AOAC Int.

Keywords: *Aflatoxin, hazelnuts, Azerbaijan`s regions*

J-3
MYCOTOXINS SCREENING IN MAIZE BY IMMUNOASSAYS: HOW EXTRACTION MIGHT AFFECT THE ACCURACY OF RESULTS

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A mycotoxin test procedure generally consists of three main steps: sampling, sample preparation and analysis. All together they lead to a global analytical error, mainly depending on the sampling step, but the sample preparation can significantly affect the accuracy of the analysis, even when a correct sampling procedure is performed. Typically, from sampling to analysis a three steps procedure is required: grinding, sub-sampling and performing the extraction. Several publications already showed interesting connections between the grinding procedure and the accuracy of the results. Fine grinding and slurry preparations are for example recommended for aflatoxins analysis in maize due to their significantly uneven distributions inside the matrix (Pannunzi et al., 2012). The aim of the present study is to focus onto an almost neglected aspect such as the effect of shaking during the extraction step. Incurred maize materials previously characterized by HPLC were analysed for deoxynivalenol and fumonisins content using two Tecna ELISA test kits, Celer DON v2 and I'screen FUMO respectively. Both test kits have the same preparation procedure for maize: weigh 5 grams of ground material, add 1 g of sodium chloride and 25 ml of a methanol: water 70: 30 mixture, shake for 3 minutes, filter. For I'screen FUMO a further dilution step is necessary. Different shakings were applied: hand shaking, vortexing, blending, magnetic stirring and horizontal shaking at 400 rpm, simulating different options that analysts could choose. Celer DON v2 test kit showed comparable accuracy when manual and horizontal shaking were implemented, while the accuracy of the analysis turned to be affected by other ways which led to overestimations. I'screen FUMO showed no significant differences between extraction procedures but a slight overestimation when using the blender. As a conclusion, this preliminary study showed that extraction may affect the accuracy of the result. In the two assays the different extraction ways have a different impact on the result; it is therefore advisable to compare the most common extraction modes when evaluating the performances of a new mycotoxin screening assay.

Keywords: *ELISA, mycotoxins, extraction mode, accuracy*

J-4
A RELIABLE AND WELL-CONTROLLED SCREENING TOOL: I'SCREEN AFLA M1 MILK ELISA KIT

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Due to high temperature and drought during summer, the maize cultivated in southern Europe is often submitted to heavy stress and consequently contaminated by the mould Aspergillus flavus. Therefore, the maize kernels could contain very high aflatoxin B1 levels (50–100 ppb). Being this cereal widely used for feeding milk cows, the concentration levels of aflatoxin M1 in milk and dairy products, could be high as well. During such crisis as well as in normal routine conditions, it is mandatory to have reliable, precise and accurate screening tools to control the aflatoxin M1 contaminations. The best choice is to use an analytical method, which is already controlled and monitored. Here, the control procedures as well as the monitoring program for I'screen AFLA M1 MILK (code MA440), a Tecna ELISA test kit for the quantitative determination of aflatoxin M1 are presented. Tecna submits every batch of this kit to an in house quality control, in terms of accuracy and precision. According to a quality control scheme, three (or more) reference or control materials are run in three replicates, in three analytical sessions. Intrabatch control is precision-based: the intrassay CV (n=3) has to be <15%, meanwhile interassay CV (n=3) has to be <20%. Accuracy is determined and given in a control flow chart. The acceptance criteria is that 68.3% of results must not exceed 1 standard deviation of the control/reference sample specifications. Since 2005, Tecna has participated in a number of proficiency schemes organized by different providers: AIA (Associazione Italiana Allevatori, Italian Breeders Association), FAPAS (UK), Progetto Trieste (Test Veritas, Italy) and Bipea (France). The mean z-score turned to be 0.66 corresponding to a mean recovery of 114±10% (n = 35, 9–400 ppt contaminated raw milk samples). The latest z-score obtained in FAPAS 2013 round for milk powder was -0.3, corresponding to a mean recovery value of 93% (FAPAS® report 04123, table 1, laboratory number 45). I'screen AFLA M1 MILK performance can be gathered in Progetto Trieste proficiency test final reports, where it is therefore possible to monitor the average performance of each test kit used by different laboratories. For instance, in 2010, in one proficiency test for the analysis of two naturally incurred materials, mean z-score of I'screen AFLA M1 MILK was 0.71 and in 2011 -0.30. As a conclusion, a critical verification of the performances of I'screen AFLA M1 MILK showed its high reliability for routine screening of aflatoxin M1 in milk.

Keywords: *Aflatoxin M1, milk, proficiency, control materials*

J-5
DEVELOPMENT AND VALIDATION OF A FAST UPLC–MS/MS METHOD FOR AFLATOXINS DETERMINATION IN PEANUTS SAMPLES

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Aflatoxins are highly toxic, teratogenic, mutagenic and carcinogenic metabolites produced by fungi of the genus *Aspergillus*, mainly by species *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. These fungi are distributed worldwide and may grow in various agricultural commodities like cereals, tree nuts and spices in the field and in the storage conditions. Methods for aflatoxins analysis in food samples usually include extraction with a mixture of an organic solvent and water and determination by direct fluorimetric measure, or injection in a chromatographic system with pre or post column derivatization step and fluorimetric detection. Analytical methods employing mass spectrometry detection for aflatoxins analysis have also been described. In this study, a fast method for determination of aflatoxins B1, B2, G1 and G2 by Ultra Performance Liquid Chromatography (UPLC) coupled to Mass Spectrometry (MS) detection was developed and validated. Results obtained for peanuts samples were compared with the official AOAC method. Finely ground peanut samples were extracted with acetonitrile/water (80:20, *v/v*) and final extracts injected into UPLC column. Experiments were performed in an Acquity I-Class UPLC system equipped with a C18 column (BEH, 2.1 × 50 mm, 1.7 µm) and coupled to a Xevo TQ-S Mass Spectrometer (Waters, MA, USA). Gradient elution was optimized with mobile phase composed by water and acetonitrile both containing 0.01% of formic acid. Total chromatographic run time was 2.5 min with flow rate kept at 0.6 mL/min. The mass spectrometer was operated in MRM mode using electrospray ionization in positive ion mode. Cone voltage, collision energy and MRM transitions (major precursor ion > fragment ion) were optimized for each compound individually. Capillary voltage, source and desolvation temperature, and cone and desolvation gas flow were optimized on the MassLynx tune page. Quantification MRM transitions were *m/z* 313>285, 314>287, 329>243, 331>245, for AFB1, AFB2, AFG1, AFG2, respectively. The LOD (signal/noise, 3:1) and LOQ (signal/noise, 10:1) estimated for AFB1 and AFB2 in sample extract were 0.03 and 0.1 ng/mL, respectively. For AFG1 and AFG2, LOD and LOQ were 0.02 and 0.07 ng/mL, respectively. Linearity was evaluated using standard solutions of aflatoxins at concentration range from 0.1 to 4 ng/mL, and all the analytes presented R^2>0.99. Recovery was estimated by fortified samples prepared in three replicates at 10, 20 and 30 ng/g of each aflatoxin. Recovery obtained varied from 71 to 90 % and coefficient of variation ranged from 0.20 to 3.8 %. Twenty three peanuts samples were analyzed by the proposed method and by the AOAC method and a significant positive correlation (r=0.93; p=0.0001) was found for the sum of aflatoxins obtained by both methodologies. The validated method is fast, selective and sensitive to determine residues of aflatoxins in peanut samples and it might be applied to other food matrices.

Keywords: *Aflatoxins, peanuts, UPLC, mass spectrometry*
Acknowledgement: *FAPESP*

J-6
SIMULTANEOUS DETERMINATION OF BEAUVERICIN AND ENNIATINS IN GRAIN BY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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In accordance with increasing concerns about food safety and global warming, contamination of mycotoxins in food and feedstuffs has gained a lot of attention. So far, researches on mycotoxins have been focused on major mycotoxins like aflatoxins, ochratoxins or traditional fusarium toxins (trichothecenes, fumonisins, zearalenones). Beauvericin (BEA) and enniatins (ENNs) are emergingsecondary toxic metabolites produced by Fusarium species such as *F. proliferatum*, *F. avenaceum* and *F. subglutinans*. These toxins were discovered relatively late and have not yet been fully elucidated.
We have developed an experimental method for analysis of beauvericin and four enniatins (enniatin A, enniatin A1, enniatin B, enniatin B1) in grain simultaneously and have set up appropriate conditions based on liquid chromatography with electrospray ionization triple-quadrupole mass spectrometry (LC/MS/MS). Homogenized grain samples were extracted with 50% methanol and cleaned up with Waters HLB cartridge.
The method's performance characteristics are specificity, linearity, limit of quantitation, recovery and precision. The specificity was examined by comparing negative matrix samples and mixed standards spiking in negative matrix (rice, barley, sticky rice, flour, corn, brown rice and sorghum) samples. No interfering signals were detected. Good linearity was obtained (r2≥0.999) within the range of 2 to 200 µg/kg. The limit of quantitation was determined at 3 µg/kg, which takes the dilution factor into account, and the ion ratio was fit to the standards ratio. The recovery was calculated at three concentrations (10, 50, 100 µg/kg) by using the matrix matched calibration curves to compensate for the matrix effect. The average recovery ranged from 73.6% to 105.0% with a relative deviation lower than 9.9% in rice and brown rice samples. The repeatability (intraday precision, five replicates at three concentrations) and the reproducibility (interday precision, five replicates at three concentrations on three different days) experiments were conducted to prove this method is fit for the purpose. This method was well applied for the determination of beauvericin and four enniatins in commonly consumed grain samples and it allows us to conduct further research about these mycotoxins' occurrence data in Korea.

Keywords: *Fusarium mycotoxin, beauvericin, enniatins*

J-7
REDUCTION OF FUMONISIN CONTAMINATION IN MAIZE BY NEAR INFRARED SPECTROSCOPY

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The European regulation CE 1126/2007 dictates the maximal mycotoxins contents allowed in cereals. The direct measurement of mycotoxins contents with the reference methods is long and tedious. Furthermore, it is destructive and cannot be used at silo. Alternative tools such as infrared spectroscopy are studied. In this study, the spectral data collected on maize kernels naturally contaminated by mycotoxins, were studied to predict mycotoxins and particularly fumonisin contents. Artificial Neural Networks (ANN) were used to reduce mycotoxin contents in lots of grains, regarding the animal and human feed and food limits.

Keywords: *Mycotoxins, cereal, infrared spectroscopy, ANN, lot*

J-8
OCCURRENCE OF MYCOTOXINS IN ANIMAL FEED FROM ORGANIC FARMING PRODUCTION

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Organic farming has been continuously growing in the Czech Republic since 1989. The crops of great importance are cereals followed by fodder crops. Organic products have been grown in compliance with the principles of organic farming that typically excludes for instance the use of artificial fertilizers, fungicides and herbicides. This fact could influence fungal growth and subsequent mycotoxin production. Also good agricultural practices like crop rotation, soil preparation, weed control, typical for organic farming, are critical management tools for minimizing mycotoxin contamination. Since 2010 Central Institute for Supervising and Testing in Agriculture has been a control authority responsible for official controls in organic farming in the Czech Republic. Feed and raw materials for feed production from organic farming are investigated for mycotoxin occurrence. The recently developed multiresidue mycotoxin method based on the unbuffered QuEChERS and ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) represents a useful tool for feed analysis. The method validated for determination of 17 mycotoxins (deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin, ochratoxin A, zearalenone, aflatoxins, fumonisins, beauvericin and enniatins) was applied to mycotoxin screening of animal feed. This study presents results of both convetional and organic feed samples produced between 2012 and 2013.

Keywords: *Mycotoxins, animal feed, organic farming*

J-9
HIGH THROUGHPUT ONLINE AUTOMATED SAMPLE CLEAN-UP APPROACH FOR DETERMINATION OF DEOXYNIVALENOL AND DEOXYNIVALENOL-3-GLUCOSIDE IN CEREAL GRAINS AND COMPARISON WITH STANDARDISED MYCOTOXIN SAMPLE PREPARATION TECHNIQUES

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Deoxynivalenol-3-glucoside (D3G) is a phase II plant detoxification product of deoxynivalenol (DON) found in naturally contaminated wheat, barley, oat and maize. Toxic effects of DON include emesis, haemorrhage, circulatory shock, resulting in implications to animal feeding behaviour and immune system function. The European Commission (EC) has set legislation limits for DON in cereal grains and cereal-based products intended for human consumption (EC No. 1126/2007). D3G on the other hand, has been found to be less toxic, both in vitro (Caco-2 and Hep-G2 cell lines) and in vivo (rats), compared to DON, however, it can be hydrolysed back to the precursor mycotoxin during digestion, fact that has to be taken seriously into consideration. Therefore, occurrence of masked mycotoxins has to be monitored and controlled to ensure food safety. In this study we developed a high throughput automated sample clean-up method for determination of DON and its masked form, D3G, and compared its performance to different sample preparation techniques commonly used in mycotoxin analyses. These included: i) extract-shoot, ii) Romer Labs MycoSep 227, iii) MycoSep 227 with additional acetonitrile elution and iv) centrifugal filtration (Millipore). Ground barley and wheat samples were spiked at 2 concentration levels (lowest and highest allowed levels in food) of DON and D3G (ratio approx. 5:1), with 6 six replicates at each level. The samples were weighed (20 g) into 250-mL Erlen-meyer flasks and spiked with DON and D3G, before extraction with 80 mL of acetonitrile:water (84:16, v/v) by shaking for 2 h. 8 mL of the raw extracts were used for each of the 5 sample preparation techniques tested. After processing samples were evaporated to dryness and reconstituted to a final volume of 500 µL of the appropriate solvent. Average recovery (RSD%) in extract-shoot for DON and D3G in barley was 94–99% (3–8%) and in wheat 87–99% (1–10%). Automated sample clean-up average recoveries were 73-96% (7–11%) and 72-102% (6–13%) in barley and wheat, respectively. Both methods showed acceptable results of recovery and repeatability according to legislation. Application of MycoSep 227 yielded comparable recoveries for DON 74–94% (3-11%) in barley and 87–105% (4–8%) for wheat, but not for D3G (recoveries < 30%). Finally, centrifugal filtration gave higher RSD% values than all other methods, ranging between 6–13%, but recoveries were within the acceptable 70–120% range. The benefit of using automated sample clean-up is the ability to inject directly pure extracts into the MS, offering faster analyses and less matrix interferences that may cause ion suppression/enhancement. Other approaches required additional steps or frequent system maintenance. To sum up, development of reliable and quick methods for masked mycotoxin determination along with the native toxins is of high importance as new masked forms are constantly being discovered.

Keywords: *Masked mycotoxins, sample preparation, deoxynivalenol, LC–MS*

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J-10
OCCURRENCE OF PATULIN IN PURE APPLE JUICE PRODUCED IN SERBIA

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Food contamination by mycotoxins is a serious problem for the food industry and for human health. Patulin (PAT) is a mycotoxin produced by some species of Aspergillus, Byssochlamys and Penicillium. Due to patulin toxicity, JECFA established a provisional maximum tolerable daily intake of 0.0004 mg/kg body weight/day. IARC has classified PAT as category 3, not classifiable regarding its carcinogenicity to humans. Apple juice is considered as major source of patulin intake in human diet. Patulin contamination is an indicator of unsound apples in juice production. The European legislation has established a maximum level of 0.050 mg/kg of patulin in apple juice, and the same restriction is valid in Serbia. This study evaluates the incidence and level of patulin in a survey of pure apple juice produced in Serbia. A total of 23 samples were acquired from supermarkets in an attempt to collect all domestic brands of pure apple juice. Analytical method was comprised of ethyl-acetate:hexane extraction, solid-phase clean-up on C18 sorbent, and HPLC-UV separation and detection. Prior to the analysis of real samples, laboratory performance of the metod was tested. Linearity of the method was studied in the range 10–1000 ng/ml, obtaining linear correlation coefficient higher than 0.999. LOD was determined as 0.0007 mg/kg, and LOQ 0.002 mg/kg. Accuracy and precision were evaluated by means of recovery experiments, analysing quintuplicate samples at 0.01, 0.025, 0.05 and 0.1 mg/kg concentration levels. The method was found to have satisfactory accuracy and precision: mean recovery 85%, precission in terms of relative standard deviation

Keywords: *Patulin, apple juice, HPLC*

J-11
AFLATOXIN M1 IN MILK AND BABY FORMULAE MARKETED IN SERBIA IN SUMMER 2013

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Aflatoxin M1 (AFM1), considered to be human carcinogen (IARC class 1), is the major metabolite of aflatoxin B1 (AFB1) in mammals, beeing partly excreted into milk. The quantity of AFM1 in the milk depends on the concentration of AFB1 in contaminated feed, with a conversion rate 0.3–6.2%. Milk is an important dairy product for human consumption, and infants are especially vulnerable population. The objective of this study was to investigate the occurrence of AFM1 in fluid milk and infant formulae on Serbian market in summer 2013, following large scale AFM1 milk crisis that started during previous winter and resulted in ten-fold increase of legal limit for AFM1 in milk, from 50 ng/kg, as in EU, to 500 ng/kg. Regarding infant formulae, maximum allowable limit in Serbia is the same as in EU (25 ng/kg). A total of 20 samples representing the fluid milk brands produced in Serbia included pasteurized milk (6), and ultra-high temperature (UHT) treated milk (14). Twenty one sample of cow's milk based commercial baby formulae belonging to the 8 producers (only one from Serbia) were classified as infant formulae (9), follow-on (8) and toddler formulae (4). The contamination with AFM1 was assessed using immunoaffinity column clean-up, liquid chromatography separation and fluorescence detection. As a confirmation of AFM1 in positive samples, TFA derivatization was carried out as described by AOAC. To assess the performance of analytical method, precision and recovery assays were carried out on AFM1-free formula, spiked with AFM1 at levels of 100, 250 and 440 ng/kg of formula powder. In the case of milk, spike levels were 50 ng/l and 500ng/l, all done in three-replicates. Acceptable recovery and precision values were obtained for both fluid milk and infant formula (mean recovery 67% and 70%, relative standard deviation 5% and 7%, respectively). Analysis of reference material met the certified concentration (440±60 ng/kg; milk powder, ERM-BD284), giving 450 ng/kg. The results of the study revealed high incidence of AFM1 in milk samples as all of them featured a contamination above the LOQ (10 ng/l), ranging from 17 ng/l to 227 ng/l. Mean level of AFM1 was 95 ng/l in both pasteurized and ultra-high temperature types of milk, with the lowest values obtained for two organic samples (17 and 27 ng/l). AFM1 levels in all investigated milk samples remain under the Serbian regulatory maximum level, but if compared to EU limit, 75% of them is on or above it. However, AFM1 levels recorded in present study are generally lower in comparison with values measured in previous winter in Serbia. Concerning infant formulae, AFM1 was found in only one sample, in quantity below the legal limit (20 ng/kg in ready to use formula). The sample was follow-on formula, produced in Serbia in February 2013. The results of present study call for further studies and actions, including stringent control of AFM1 in the food supply.

Keywords: *Aflatoxin M1, milk, baby formulae, HPLC*

J-12
DEVELOPMENT OF OPTIMIZED MULTICLASS CLEAN-UP METHODS FOR LC–MS/MS ANALYSIS OF MYCOTOXINS IN ANIMAL FEEDSTUFFS INCORPORATING A NOVEL SPE COLUMN

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Mycotoxins are a group of toxic and often carcinogenic or genotoxic metabolites produced by several strains of fungi found on food and fodder crops worldwide. Mycotoxins have great potential to cause harm to humans, crops and farmed animals. As a result, a wide range of food and feedstuff substrates require testing for mycotoxin contamination. The diversity of analyte structure and food substrate generates a significant analytical challenge. Traditionally, mycotoxins have been analyzed using multiple methods, each optimized for a single mycotoxin or group of closely related toxins. Multi-analyte approaches are being made increasingly possible by the adoption of liquid chromatography-tandem mass spectrometry (LC–MS/MS) based analyses. Due to the selective nature of LC–MS/MS, highly selective sample preparation techniques are often no longer essential to meet the residue limits required. However, appropriate sample preparation remains necessary in order to min imize matrix effects and maximize assay robustness. Here we present work on the development of simple catch-and-release sample preparation methods using a novel solid phase extraction (SPE) column for a variety of mycotoxin classes commonly encountered in animal feed providing cleanup for multiple mycotoxins: aflatoxin B1, ochratoxin A, fumonisin B1, T-2 toxin, HT-2 toxin, zearalenone and deoxynivaenol. The developed methods were successfully applied to the analysis of mycotoxins in soya and compound animal feed substrates. We are able to demonstrate methods capable of reducing matrix effects to levels that enable simultaneous measurement with a targeted LC–MS/MS method demonstrated by analyte signal/noise >10:1 at LOQ. Our methods demonstrate linear responses (r^2 > 0.99) over the working range. We demonstrate determination of 7 mycotoxins at or below regulatory requirements of current EU and US legislation with recoveries of between 70 and 110% at LOQ.

Keywords: *Mycotoxins, LC–MS/MS, SPE, multiclass, animal feed*

J-13
DEVELOPMENT OF OPTIMIZED MULTICLASS MYCOTOXIN CLEAN-UP METHODS FOR LC–MS/MS ANALYSIS USING A NOVEL SPE COLUMN

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Mycotoxins are a group of toxic and often carcinogenic or genotoxic metabolites produced by several strains of fungi found on food crops worldwide. Mycotoxins have great potential to cause harm to humans, crops and farmed animals. As a result, a wide range of food and feedstuff substrates require testing for mycotoxin contamination. The diversity of analyte structure and food substrate generates a significant analytical challenge. Traditionally, mycotoxins have been analyzed using multiple methods, each optimized for a single mycotoxin or group of closely related toxins. Multi-analyte approaches are being made increasingly possible by the adoption of liquid chromatography-tandem mass spectrometry (LC–MS/MS) based analyses. Due to the selective nature of LC–MS/MS, highly selective sample preparation techniques are often no longer essential to meet the residue limits required. However, appropriate sample preparation remains necessary in order to minimize matri x effects and maximize assay robustness. The aim of our work was to develop a polymeric resin capable of isolating multiple classes of mycotoxins from a range of food and feed substrates. This was achieved by means of a screening program incorporating a library of diverse polymeric base scaffolds. Screening this library yielded a number of candidate resins. Subsequent optimization of the candidate resins produced a novel resin that provided the broadest, most efficient selectivity for maximised performance. Using the resulting resin we developed simple catch-and-release SPE methods for the selective extraction of mycotoxins from: grain (wheat, maize, barley); nuts (Brazil nut, peanut); animal feed (soya, compound feedstuff); apple juice and infant foodstuffs (formula and cereals). We were able to demonstrate robust and reproducible extraction of multiple mycotoxins: aflatoxins, ochratoxin A, type 1 and 2 trichothecenes, ergot alkaloids, fumonisin B1 and zearalenone from the above matrices by selective modulation of: liquid extraction, resin equilibration, sample loading, wash and elution conditions. Each of these will be discussed in detail. Quantitation was by the use of one of two HPLC–MS/MS platforms dependent on the substrate extracted: a Waters 2795 and Quattro Ultima Pt or Shimadzu Nexera LC-30 and AB Sciex Triple Quad 5500. Here we present work on candidate resin evaluation and demonstrate sample preparation methods that give quantitative assays for multiple mycotoxins from a variety of matrices over three orders of magnitude linear range with correlation coefficients >0.990. LOQ will be demonstrated at or below current EU MRL for the relevant matrix/mycotoxin combination with S/N >10:1 and reproducibility below specified limits. In the majority of cases recoveries were between 70% and 110%, exceptions will be discussed. The sample preparation methods developed were compared using matrices supplied by FAPAS with known determinand levels and their performance will be presented.

Keywords: *Mycotoxins, LC–MS/MS, SPE, multiresidue*

J-14
APPLICATION OF TLC FOR SCREENING TOXIGENIC MICROMYCETES ISOLATED FROM CANNED FOOD AND OBJECTS OF INDUSTRIAL ENVIRONMENT OF BELARUSIAN CANNERIES

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The aim of the study was selection of toxigenic strains among fungal microbiota of canned food and objects of industrial environment of canneries by TLC.

The objects and methods of researches. 67 cultures of micromycetes isolated from fruit and vegetable canned food for children and the air and the surfaces of process equipment were object of researches. Fungal secondary metabolites obtained by incubation on the medium Chapek-Dox with yeast extract and sucrose at a temperature (24±1)°C for 10 days and extracted by chloroform. Each fungal extract was chromatographed in seven solvent systems [1] on Silufol sheets. The Silufol sheets were detected under UV light at a wavelength of 254 nm. Preliminary identification of mycotoxins was performed by comparing the Rf values and color of spots of the investigated fungal secondary metabolites in seven systems with Rf values and color of spots of known mycotoxins in these systems [1].

The results of researches. More than half of fungal strains secreted in cultural medium substances that fluoresced under UV light. We have not received a complete coincidence of «chromatographic spectra» of investigated secondary metabolites and known mycotoxins. For further study we selected the fungal secondary metabolites at which there were spots coinciding in Rf values and color in four solvent systems with Rf values and color of spots of known mycotoxins in these systems.

Conclusions. The TLC could be used for preliminary screening of toxigenic strains micromycetes, but it is desirable to use the standards of mycotoxins.

[1] Ďuračkovâ, Z. Systematic analysis of mycotoxins by thin-layer chromatog-raphy / Z. Ďuračkovà, V. Betina, P. Nemec // Journal of Chromatography. – 1976. – v. 116. – P. 141–154.

Keywords: *Fungal microbiota, mycotoxins, TLC*

J-15
THE FUTURE IN MULTI-MYCOTOXIN ANALYSIS – MYCOSPIN™400 CLEAN-UP AND STABLE ^{13}C-LABELED INTERNAL STANDARDS IMPROVE ACCURACY AND SENSITIVITY IN MYCOTOXIN LC–MS/MS METHODS

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The need for multi-mycotoxin analyses is constantly rising and the technology of choice is LC–MS/MS. Laboratories are increasingly using LC–MS/MS methods in their routine testing operation. Nevertheless, a problem with LC–MS/MS can be interferences from matrix components leading to differences in analyte ionization. The application of fully ^{13}C-labelled internal standards will correct such mass signal intensities between various sample matrices and pure standard calibrants to ensure qualified analysis results. There are several advantages over alternatives like deuterated (^{2}H) internal standards. Replacing ^{12}C by ^{13}C changes the total mass of the atom only slightly, while using deuterium, the mass doubles, thus, ^{2}H labeled mycotoxins might show retention time shifts, resulting in less accurate LC–MS/MS results. Nowadays, highly sensitive mycotoxin detection methods are demanded by EU legislation and subsequently by the mycotoxin testing food and feed safety market. For being able to detect multiple mycotoxins at such low detection limits a sample cleanup step should be implemented in the LC–MS/MS method. A novel rapid multi-mycotoxin cleanup method to increase the LC–MS/MS method sensitivity was developed by Romer Labs. The use of ^{13}C isotope-labeled internal standards in conjunction with the MycoSpin™ 400 Multitoxin Clean-Up Column allows for a method which is applicable to analysis of a wide variety of matrices, with no limitations by molecular mass, and a straightforward sample preparation. This poster will show the different procedures of how to use ^{13}C-labeled internal standards. Furthermore, it will illustrate how important it is to apply internal standards when running quantitative mycotoxin analyses on an LC–MS/MS system. And finally a new multi-mycotoxin cleanup method for a better LC–MS/MS sensitivity on complex sample matrices will be presented.

Keywords: *LC–MS/MS, Multi-Mycotoxin Analysis, ^{13}C labeled internal standards, Multitoxin Clean-up column, Column*

J-16
LATERAL FLOW DEVICES: AGRASTRIP® WATER BASED EXTRACTION FOR MYCOTOXIN ANALYSIS

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Rapid tests are quick and easy to perform test kits that can produce qualitative as well as quantitative test results. One-step lateral flow immunochromatographic assays, based on a competition immunoassay format, are used if quick test results are needed, i.e. in remote areas and therefore test systems for field use are essential. In mycotoxin testing of food and feed samples quick decisions can be necessary and therefor Lateral Flow Devices are often the method of choice. Beside rapid solutions for testing mycotoxins, the market desire for more sustainable and less toxic extraction methods is gaining more and more attention. Nowadays developments in mycotoxin testing are going forward to develop quick solutions, but also considering resources and environmental concerns. The water based extraction is a simple handshaking extraction method, but less toxic than other extraction methods. No further use of organic solvents, such as methanol and acetonitrile, is necessary, because of the fact that they can be replaced by an aqueous buffer system. Advantages of Lateral Flow Devices for mycotoxins using water based extraction are easy handling, rapid test results, and a test system which is less toxic, environmental and user friendly. So far the water based extraction method was developed for aflatoxin and zeraleone (ZON) test strips, and will be continued for other regulated mycotoxins. Calibration curves will be presented and accuracy and precision data will be provided.

Keywords: *Lateral Flow Devices, Water based extraction, Mycotoxin Analysis, Rapid Test, Simple Extraction*

J-17
DEVELOPMENT OF RAPID QUANTITATIVE DIPSTICK TESTS FOR FUMONISINS, AFLATOXINS AND DEOXYNIVALENOL IN GRAINS

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Mycotoxins are toxic natural secondary metabolites produced by several species of fungus, such as for example Aspergillus and Fusarium species, on agricultural commodities in the field or during the storage. Among these mycotoxins, Fumonisins (FUMs), Aflatoxins (AFLs) and Deoxynivalenol (DON) can have hazardous effects on the human and animal health if they are consumed at higher levels than the European regulations (EC No 1881/2006) or at lower levels but in association with other mycotoxins. Indeed, it is now well known that all these toxins are immunotoxic, carcinogenic, hepatotoxic and neurotoxic. Therefore, the monitoring of mycotoxins in food and feed is primordial. For that purpose, different methods could be used as confirmatory methods such as HPLC and LC–MS or as screening methods like ELISA and lateral flow devices (LFDs). The use of screening methods allows high throughput, cost-efficient analyses and on-site measurements that could be performed by unskilled people. LFDs for mycotoxins detection in food and feed have recently been developed in the last five years. The first generation of dipstick tests devoted to the mycotoxins detection was qualitative tests giving a positive or negative response. To date, farmers and grains collectors want first to know if their commodity is contaminated in mycotoxins but also want to know the exact level of this contamination for financial and applications purposes. Therefore, Unisensor has decided to develop a wide range of quantitative lateral flow devices for the most relevant mycotoxins. In this work, we are presenting the new developments of these quantitative LFDs especially for FUMs, DON and AFLs detection in different commodities. Fumosensor®, Donsensor® and Aflasensor® Quanti have been developed for the respective quantification of Fumonisins (B1, B2 and B3), DON and Aflatoxins (B1, B2, G1, G2) in different commodities. These tests are supposed working with an optical reader (Readsensor®) which translates the test line intensities into mycotoxin concentrations thanks to pre-established calibration curves. Mycotoxins are recovered from the commodity by a simple vortex mixing in alcoholic media during 2 minutes. The dipstick tests are run in only 5 minutes. Quantification ranges for FUMs (200 to 10000 µg/kg), DON (200 to 3000 µg/kg) and for AFLs (2 to 60 µg/kg) have been determined. These ranges can be further extended by a simple extract dilution. The accuracy of Fumosensor®, Donsensor® and Aflasensor® Quanti has been analyzed by comparing LC–MS results with results issued from dipstick analyses on reference contaminated materials. Coefficients of variation less than 18% have been calculated showing the "fit for purpose" character of these new quantitative dipstick tests as screening techniques.

Keywords: *Fumonisins, Aflatoxins, Deoxynivalenol, quantitative dipstick*

J-18
DEVELOPMENT OF THE FIRST QUANTITATIVE MULTIPLEX DIPSTICK FOR THE SIMULTANEOUS DETECTION OF DON AND ZON MYCOTOXINS IN GRAINS

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Zearalenone (ZON) and deoxynivalenol (DON) are two mycotoxins commonly associated to cereal products such as wheat, maize, oat and barley and feed. They are mainly both produced by Fusarium graminearum fungus explaining their co-occurrence in certain type of food and feed. DON and ZON are recognized having respectively neurotoxic and oestrogenic effects on the human health in addition of their well-known immuno-suppressive properties. Due to their hazardous consequences to human health, their presence in food has been regulated through EU regulations (EC No 1881/2006). Their occurrence in cereals and in cereal-based products is usually monitored with confirmatory methods such as HPLC or LC–MS. These methods are reliable and give accurate quantitative results. Anyway, they are very expensive, they need of skilled people and do not allow field measurements. The necessity for farmers and grain collectors in having fast detection methods being accurate, inexpens ive and allowing on-site measurements has prompted us to develop lateral flow devices (LFDs) for the monitoring of mycotoxins in food and feed. In this work, we are presenting the very first development of a quantitative dipstick test allowing the simultaneous detection of DON and ZON. This new development represents for the end-user a time saving since only one extraction experiment is needed to recover both toxins and only one dipstick test gives a quantitative response of the contamination in each mycotoxin. The 2-Mycosensor® dipstick test is a multiplex dipstick test developed by Unisensor for the simultaneous quantification of DON and ZON mycotoxins in raw cereals and feed. This test is made of two test lines respectively for DON and ZON and one control line. Each test line is analyzed by an optical reader (Readsensor®) and translated into a DON and ZON concentration thanks to pre-established calibration curves saved in this reader. DON and ZON are both extracted with an alcoholic medium for 2 minutes with a vortex mixer. Recoveries higher than 80% have been measured for all matrices. The dipstick test only takes 5 minutes. Six dipstick tests can be run at the same time, making possible the analysis of DON and ZON concentrations contained into 6 samples in less than 20 minutes, extraction time included. Limits of quantification of 200 µg/kg for DON and 50 µg/kg for ZON have been determined and quantification ranges going from 200 to 3000 µg/kg for DON and from 50 to 750 µg/kg for ZON have been measured without any further sample extract dilution. These two quantification ranges can be extended by a simple extract dilution. The accuracy of the 2-Mycosensor method has been analyzed by comparing LC–MS results with results issued from 2-Mycosensor on reference contaminated materials. Coefficients of variation included between 8 and 17% have been calculated showing the "fit for purpose" character of this new multiplex quantitative dipstick test as a screening technique.

Keywords: *Multiplex, zearalenone, deoxynivalenol, dipstick*

J-19
VALIDATION STUDY OF A RAPID QUANTITATIVE DIPSTICK TEST FOR AFLATOXIN M1 IN MILK

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Aflatoxin M1 (AFM1) is one of the major Aflatoxin B1 (AFB1) metabolites excreted in mammalian systems in milk, urine and animal serum. Like its parent compound, AFM1 keeps its carcinogenic, mutagenic and toxic effects. In lactating animals (cow, sheep, goat), AFM1 appears in milk at levels proportional to the AFB1 contamination level of the feed. Around 5% of the AFB1 contamination is converted into AFM1 in milk after 12 to 72 hours. AFM1 is chemically highly stable, no particular treatment allows the reduction of its contamination. Therefore, its monitoring in milk products remains the only effective way to avoid its hazardous effects on the human health. In line with this, the European commission decided to regulate the occurrence of AFM1 in raw and UHT milk at 50 ppt (EC 1881/2006). The official techniques used to monitor the AFM1 level in milk are HPLC and LC-MS. The price, the complexity, the low output and the need of trained people for this technique s have forced the scientific community to develop rapid and accurate low cost methods making possible the screening of milks directly at the farm level. In line with these requirements, Unisensor has made available on the market its AFLASENSOR QUANTI, a rapid quantitative lateral flow immunoassay able to quantify AFM1 in raw cow, goat and sheep milks. This dipstick test quantifies AFM1 from 20 to 150 ppt without any milk treatment in only 10 minutes running time. AFLASENSOR QUANTI has been internally validated in raw cow milk following the EURACHEM guidelines in a 3-day study on three different batches of kits. This study showed that Detection limit is less than 20 ppt while the limit of Quantification is 25 ppt. Depending on the detected AFM1 concentration, relative standard deviations ranging from 8 to 21% in repeatability conditions and from 12 to 23% in reproducibility conditions have been determined. Trueness and precision study showed good accuracy of the results obtained with Aflasensor. As a matter of fact, percentages of Trueness and Precision, respectively, ranging from 80 to 100% and 75 to 90% have been calculated depending on the AFM1 concentration. Dipstick performances have been evaluated on goat and sheep milks, this study showed that with a simple arithmetic extension of the cow milk result, the quantification of AFM1 from 20 to 50 ppt in these two matrices is possible without any pre-treatment of the milk.

Keywords: *Aflatoxin M1, lateral flow immunoassay, milk*

J-20
EVALUATION OF EMERGING FUSARIUM MYCOTOXINS BEAUVERICIN, ENNIATINS, FUSAPROLIFERIN, AND MONILIFORMIN IN DOMESTIC RICE IN IRAN

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The occurrence of emerging Fusarium mycotoxins beauvericin, enniatins (A, A1, B, B1, B2, B3) fusaproliferin and moniliformin was evaluated by a liquid chromatography/electrospray ionization-tandem mass spectrometric (LC/ESI–MS/MS) technique in 65 domestic rice samples produced in Gilan and Mazandaran Provinces in Iran. The results showed that 46% of the samples were contaminated with at least one of the emerging mycotoxins. BEA was the most prevalent mycotoxin, which was found in 26 out of 65 rice samples at the concentrations up to 0.47 µg/kg. ENNA1 which was the only member of ENNs was detected in the samples, occurred in 6.15% of samples with an average level of 0.06 µg/kg. No detectable level of fusaproliferin and moniliformin was found. This paper reports for the first time the contamination of domestic rice samples in Iran with the emerging Fusarium toxins.

Keywords: *Beauvericin, enniatins, fusaproliferin, Iran, rice*

J-21
DETERMINATION OF MYCOTOXINS IN DOMESTIC RICE IN IRAN USING LC–MS/MS

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In this study, a liquid chromatography/tandem mass spectrometry (LC–MS/MS) method was applied for the simultaneous detection and quantification of a broad spectrum of mycotoxins and fungal metabolites in domestic rice in Iran. A total of 20 fungal metabolites were detected in 65 rice samples. The result showed that all of the samples were contaminated to at least one mycotoxin. The most prevalent fungal metabolites were brevianamide F (81.5%), emodin (46.1%), and tryptophol (43.1%). The occurrence of aflatoxin B1 (AFB1), ochratoxin A (OTA), zearalenone (ZEA), and fumonisin B1 (FB1) was 21.5%, 4.6%, 29.2%, and 9.2%, respectively. No detectable level of deoxynivelenol was found in any of the samples. The mean of regulated mycotoxins was lower than the maximum tolerable limit. Co-occurrence of AF1-ZEA, ZEA-OTA and FB1- ZEA were observed in 4.6%, 3.1% and 4.6% of samples, respectively. In addition, for the first time, mycotoxins including alternaria metabolite, citrinin, tryptophol, and kojic acid were reported in domestic rice in Iran.

Keywords: *Mycotoxin, Co-occurrence, Liquid chromatography/ tandem mass spectrometry (LC–MS/MS), Rice, Iran*

J-22
DETERMINATION OF METABOLITES FORMED BY ENZYMATIC DEGRADATION OF ERGOT ALKALOIDS

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Ergot alkaloids are one major group of mycotoxins that are mainly produced by fungi of the genus Claviceps or by grass endophytes like Neotyphodium. The occurrence of ergot alkaloids due to infections on grains and grasses has increased during the last years and remains a problem in animal nutrition. One strategy for elimination is the use of feed additives that contain microorganisms or enzymes capable of degrading ergot alkaloids. An ergopeptine-degrading Rhodococcus erythropolis strain was isolated from soil and the involved enzymes were identified. The α/β hydrolase ErgA catalyses the degradation of different ergopeptines to ergine. The amidase ErgB is responsible for the metabolisation of ergine to the end product lysergic acid. The aims of this work were the structure elucidation and determination of intermediate and final products formed during degradation of different ergopeptines by the purified enzyme ErgA, the Rhodococcus erythropolis strain and its lysate. An LC–MS/MS method for the analysis of the different ergopeptines, their corresponding epimers and the metabolites was developed on a 4000 QTrap system (AB Sciex). Investigation of the MS parameters of the known compounds (ergine, ergocornine, ergocristine, ergocryptine, ergosine, ergotamine, ergovaline, lysergic acid, and the epimers) was achieved by syringe pump infusion of single analyte solutions and software controlled optimisation. In addition, LODs and LOQs were determined. Measurements of the degradation experiment samples in different measurement modes resulted in information about molecular weight and fragmentation of the newly formed metabolites. Two main metabolites were isolated by preparative HPLC. 1H-, 13C- and 2D-NMR as well as accurate mass measurements (6550 iFunnel QTOF, Agilent Technologies) of the isolated metabolites were conducted for structure elucidation. In addition, the optical rotation was measured to determine the stereochemistry and the purity was assessed by measuring UV absorbance. During microbial and enzymatic degradation of the used ergopeptines the formation of two main groups of metabolites were observed: diketopiperazines (DKPs) and ergine hydroxy carboxylic acids. DKPs are cyclic dipeptides which are probably non-toxic. The ergine hydroxy carboxylic acids still contain the ergoline ring system, are very unstable and degrade spontaneously to ergine. Accurate mass measurements confirmed the expected sum formulas of all formed metabolites and the chemical structures were obtained by assignment of the resulting fragments in the MS/MS mode. To conclude, an analytical method for the determination of ergopeptines and their ErgA mediated degradation products has successfully been developed. Structure elucidation of the metabolites was needed to explore the mechanism of the degradation. The analytical method will be applied for monitoring the enzymatic reaction and the isolated metabolites will be used for studies on enzyme kinetics.

Keywords: *Mycotoxins, enzymatic degradation, HPLC–MS/MS*

J-23
STUDY OF AFLATOXINS INCIDENCE IN COW FEED AND MILK IN SERBIA

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A typical weather and climate conditions during the spring and summer 2012 were assumed to be the main reason for the aflatoxins contamination of corn crops in Serbia. High humidity in spring, and summer temperatures above the average contributed to the increased possibility of mycotoxins occurance in cereals in the fields. As a consequence, at the begining of 2013 contaminated corn used for dairy cows diet had negative impact on the safety of cow milk. The routine laboratory control data revealed an increased content of aflatoxin M1 in milk samples. Hundreds of raw milk and different feedstuff samples were collected during February and March 2013 and were analysed for the presence of aflatoxin M1 (AFM1) and total aflatoxins, respectively. The collected samples were a part of enhanced self-control plans of the large dairy farms. Quantitaive sandwich and competitive types of ELISA tests were used for the screening analysis of the milk and feed samples. Confirmation of the positive results obtained by ELISA tests was performed by LC–MS/MS methods. Out of 109 screening positive samples, only 17 (15.6%) contained AFM1 below the MRL of 0.05 µg/kg (Comm. Regulation EC No1881/2006). Feed used for diet of animals that produced milk with low AFM1 content were also analysed, and presence of total aflatoxins was established in 33 (67%) feed mixture samples, with the content exceeding MRL (0.01 mg/kg). Aflatoxin M1 content in the 92 positive milk samples ranged from 0.051–0.661 µg/kg. The corresponding feed samples of sunflower meal, hay silage, corn silage and sugar beet pulp were screening negative, with the content of total aflatoxins less than 2 µg/kg. The main source of aflatoxins were corn, wholemeal and feed mixtures derived from contaminated corn. In the 92 tested milk samples only one contained AFM1 in amount below the MRL (0.037 µg/kg), although the corresponding content of total aflatoxins in the feed mixture was 0.03 mg/kg, above the MRL value. In 23 positive milk samples AFM1 content ranged from 0.053 to 0.647 µg/kg, while the feed mixture samples related to these milk samples were contaminated with total aflatoxins in the amounts less than MRL and even less than detection limit of the methods. Although relatively weak correlation factor value (R=0.34) between feed and milk contamination was calculated, it is noticeable that aflatoxin content in feed exceeding MRL, led to an increased amount of AFM1 in raw milk.

Keywords: *Aflatoxins, feed, milk, ELISA, LC–MS/MS*

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J-24
DETERMINATION OF NATURALLY-OCCURRING ALKALOIDS IN FEED BY UPLC–MS/MS

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Alkaloids, which are known as secondary metabolites, are produced by a large variety of organisms, including plants, fungi, bacteria and animals. Alkaloids are not crucial for the survival of the mentioned organisms, but mainly for their protection. They are grouped by typical structural characteristics into large families such as pyrrolizidine alkaloids (PAs) comprising more than 350 individual heterocyclic compounds. As other alkaloids PAs can cause severe human and animal health problems. Intoxications caused by PAs are described by hepatotoxicity with tendency to hemorrhagic necrosis and veno-occlusion in liver, pneumotoxicity, carcinogenity, mutagenity, and genotoxicity [1]. Thus, there is a need for methods that allow to determine these dangerous plant toxins. In this study, fast, reliable and sensitive approach is proposed allowing simultaneous screening, identification and quantification of PAs in feed samples. Selected PAs representatives, namely monocrotaline, senkirkine, senecionine, seneciphylline and retrorsine, were determined by ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS). Sample preparation is based on a modified QuEChERS approach. Mean recovery, precision, matrix effects and limits of quantification were assessed for four matrices within the method validation. The presented method was applied to various feed samples of Czech production, which were expected to contain PAs. Seneciphylline was found as the most abundant, while monocrotaline, retrorsine, senecionine and senkirkine were determined in lower quantities. In general, feed samples with high content of chlorophyll showed the highest PAs levels. In addition, feed can be a source of other natural toxicants, which may affec t health conditions of livestock. Thus, ergot alkaloids have been added into the presented method and validated pursuant to 2002/657/EC [2]. This up-to-date method for the simultaneous determination of the pyrrolizidine and ergot alkaloids has been applied to selected feed materials.

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[2] 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

Keywords: *Alkaloids, feed, UPLC–MS/MS*

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J-25
DEVELOPMENT AND VALIDATION OF A QUECHERS BASED LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF ALTERNARIA-TOXINS IN TOMATO BASED PRODUCTS

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Different food and feedstuffs can be affected by moulds and, therefore, could contain mycotoxins endangering human or animal health. These moulds include the genus Alternaria (black moulds) which preferably infects cereals, vegetables and fruits. In 2003, the BfR evaluated Alternaria toxins in foodstuffs with regard to a potential risk for human health. The current scientific opinion expressed most recently by EFSA in 2011 is that the black moulds can also produce mycotoxins which can cause various toxic effects in humans and animals. However, until now the chemical structures of approximately 30 Alternaria toxins are known and toxicological data is available for only 7 toxins. At present, there is a paucity of data concerning the occurrence of Alternaria toxins in food and feedstuff. Therefore, their behaviour in the food chain and their effect on human health can hardly be assessed. The analysis of Alternaria toxins is currently lacking some commercially available standard substances and standardised validated analytical methods. At the BfR a reliable method for the analysis of Alternaria mycotoxins in foodstuffs is being developed. A QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) based extraction procedure was applied for the determination of Alternaria toxins in tomato-based products. High-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) was used for the identification and quantification. Different extraction and clean-up procedures were studied and optimized in order to obtain the best recoveries. Matrix matched calibration curves/standard additions were used to compensate for significant matrix effects. Electrospray ionization in negative mode was applied to simultaneously detect tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN) and altenuene (ALT) in a single run time of less than 10 minutes. Other Alternaria toxins such as altertoxin I (ATX I) and altertoxin II (ATX II) are also under investigation. The method will be validated and used for the detection and quantification of Alternaria toxins in commercially available tomato based foodstuff.

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Keywords: *Mycotoxins, alternaria, QuEChERS, LC–MS/MS*

J-26
BINDING AFFINITY STUDIES OF MYCOTOXIN APTAMERS USING DIFFERENT ANALYTICAL APPROACHES

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Aptamers are recognition elements representing an alternative class of receptor that are used in a broad range of analytical applications, including affinity chromatography, lateral flow devices and biosensors. Aptamer selection is performed in vitro using the Systematic Evolution of Ligands by EXponential enrichment (SELEX) process. Recently, DNA aptamers for ochratoxin A, fumonisin B1 and aflatoxin B1 have been selected, and in the case of ochratoxin A (OTA) and fumonisin B1 (FB1) different sequences have been reported. The development of an aptamer-based technology for mycotoxin determination requires a thorough understanding of aptamer-mycotoxin binding affinity that could be done using several different techniques. The binding affinity of five different OTA aptamers was investigated by equilibrium micro-dialysis, fluorescence polarization and ultrafiltration, with mycotoxin and aptamer free in solution, and by affinity chromatography, with OTA immobilized to a solid support matrix The dissociation constant (Kd) values were estimated by performing a saturation binding isotherm based on the titration of a constant concentration of OTA with an increasing concentration of aptamer. The effect of heating aptamer at 90°C before incubation with OTA was also investigated. Two of the five OTA aptamers showed Kd values in the nanomolar range using equilibrium micro-dialysis, fluorescence polarization or ultrafiltration. Among these techniques, fluorescence polarization resulted to be the most sensitive and promising one. The affinity chromatography approach was unsuitable because no plateau was reached and incomplete binding curves were obtained, indicating that immobilization of OTA may have aff ected binding properties of the aptamer. No binding was observed for the other three aptamers in none of the tested conditions. In the case of FB1 three sequences were tested by DNA footprinting, micro-dialysis and affinity chromatography. A high variability of Kd values was observed, with DNA footprinting resulting the most sensitive and promising approach, whereas no binding was observed when micro-dialysis was used. These findings indicate that before developing an aptamer-based technology careful consideration should be given to the sensitivity and working conditions of the used approach.

Keywords: *DNA-aptamer, ochratoxin A, fumonisin B1, aptamer-mycotoxin binding affinity*

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J-27
DEVELOPMENT AND APPLICATION OF AN EXACT MASS LC–MS/MS LIBRARY FOR THE SCREENING OF MYCOTOXINS AND FUNGAL METABOLITES IN FOOD AND FEED

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Mycotoxins are secondary fungal metabolites capable of causing various toxic effects including hepatotoxicity, mutagenicity, carcinogenicity or estrogenicity. Due to the complexity of food and feed samples on the one hand and the increasing number of relevant analytes (e.g. masked and emerging mycotoxins), on the other hand, accurate mass screening for food contaminants is of growing interest. While only a limited number of standard substances is commercially available, unambiguous identification of mycotoxins is required in order to avoid false positive results. Therefore, we show the development of an exact mass LC–MS/MS library and its successful application for the putative identification of various mycotoxins in food and feed samples. Library spectra for approximately 200 mycotoxins and other fungal metabolites were acquired at three collision energies in positive, negative or even both electrospray ionisation modes by injecting individual standards. The obtained MS/MS spectra were corrected to their theoretical exact m/z using a probabilistic approach based on the molecular formula and structure, resulting in an exact mass MS/MS library. Several food and feed samples were extracted with acidified aqueous acetonitrile and injected into the UHPLC–QTOF system without any clean-up. A generic water-methanol gradient was applied for the separation of the analytes within 25 minutes. The QTOF system was operated in the TOF and MS/MS mode using targeted and data dependent acquisition as well as the “All Ions MS/MS” mode. The accurate mass screening for mycotoxins and other fungal metabolites as well as confirmation of the identified contaminants by MS/MS library searching were validated by the analysis of spiked food and feed samples. High library match scores were observed even at low spiking levels. While in the TOF screening of routine samples, several additional suspects of contaminants were found. Applying the exact mass library to the MS/MS data helped to efficiently eliminate false positives. Alternatively the “All Ions MS/MS” acquisition was applied, which used fragmentation without precursor selection. The presence of mycotoxins was confirmed by the co-elution of characteristic fragment ions. Concluding, accurate mass screening using a UHPLC–QTOF in combination with an exact mass library is a powerful technique for routine screening and confirmation of a wide range of mycotoxins in food and feed samples.

Keywords: *Mycotoxins, HR–MS/MS library, screening*

J-28
THE OCCURRENCE OF MYCOTOXINS IN CORN PRODUCTS FOUND IN RETAIL STORES IN THE USA

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Mycotoxins are secondary metabolites produced by mold that are capable of causing disease and death in humans and animals. They are proven potent carcinogens and are regulated in many countries including both the U.S. Food and Drug Administration and the European Union. A limited survey (139 samples) was conducted of various corn based foods, including infant foods that were purchased at retail stores from 17 different states in the USA. These samples were analyzed to determine the occurrence of aflatoxins, deoxynivalenol, and fumonisins which are the most commonly found mycotoxins in corn. Detectable concentrations of aflatoxns, deoxynivalenol, and fumonisins were found in 44.5%, 44.5%, 74.5% respectively in the regular corn based food and 89.6%, 65.5% and 89.6% respectively in infant foods. These results demonstrate the significant 2013 occurrence of mycotoxins found in corn based foods in the USA.

Keywords: *Mycotoxins, corn, survey*

J-29
EVALUATION OF A SINGLE EXTRACTION METHOD FOR USE IN MULTI MYCOTOXIN ANALYSIS

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The use of a single extraction method for the analysis of multi mycotoxins within a single sample can be problematic. Typically at least two separate solvents are required to extract aflatoxins, fumonisins, zearalenone, ochratoxin, deoxynivalenol, T-2 toxin and HT-2 toxin from a matrix. An evaluation was performed to determine if a single extraction solvent could be utilized to efficiently extract the most commonly analyzed mycotoxins. Naturally contaminated mycotoxin reference materials were extracted with various ratios of solvents to determine the one solvent that would extract all the major mycotoxins with the highest recoveries. Acceptable extraction efficiency for all the mycotoxins were obtained using the extraction solvent of acetonitrile/water (80/20).

Keywords: *Mycotoxins, extraction*

J-30
DETERMINATION OF MYCOTOXIN RESIDUES IN BEER BY UHPLC–HR–MS AND LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION (LC–FL) IN COMBINATION WITH SOLVENT MICROEXTRACTION

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The aim of this study was to introduce a multi-mycotoxin method for the analysis of deoxynivalenol (DON), deoxynivalenol-3-glucoside, zearalenone (ZON), a-zearalenol (a-ZOL) and β-zearalenol (β-ZOL) in beer, based on a very simple and rapid sample preparation. Mycotoxins are the toxic secondary metabolites, produced by many species of microscopic filamentary fungi, occurring on field cereals, including barley. The carryover of several mycotoxins from malted grains into beer is well documented, the main objective of the present study is focused on ZON, a mycotoxin with perceived endocrine disrupting action. Endocrine disruptors are chemicals that interfere with endocrine (or hormone system) in animals, including humans. These disruptions can cause cancerous tumors, birth defects, and other developmental disorders. Zearalenone (ZON) is described chemically as a phenolic resorcyclic acid lactone and can be produced by a number of species of Fusarium. These species are known to colonise cereals and tend to develop particularly during cool, wet growing and harvest seasons. A liquid phase microextraction method was developed to study the occurrence of the target analytes as well as their metabolites in beer samples based on extraction and two phases' separation process by the assistance of magnetic stirring. Varieties of experiment factors that could affect the extraction process were optimized. The analytical methodology was validated according to the Commission Decision 2002/657/EC, using high-resolution Orbitrap® mass spectrometry ((U)HPLC–HR–MS) LC–MS/MS for confirmatory and LC–FL for quantitative purposes. The extraction method, combined with sensitive analytical techniques, leads to satisfactory reliability, sensitivity, with low consumption of reagents, and therefore is suitable for the monitoring of multi-mycotoxin residues in beer samples.

Keywords: *UHPLC Orbitrab MS, Mycotoxins, Beer, Endocrine Disruptor, Liquid Microextraction*

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J-31
DETERMINATION OF FREE AND BOUNDED FUMONISINS IN FOOD PRODUCTS BY LIQUID CHROMATOGRAPHY–ION TRAP–MASS SPECTROMETRY

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The bounded fumonisins may occur as conjugates with food macro components like carbohydrates or proteins and are found mainly in corn grain and corn based products. The concentration of these compounds may be even higher than concentration of their free analogues. This could result in exceeding the maximum allowable limits set by the various regulations. In this work a method of determination of free and bounded fumonisins (FB1, FB2 and FB3) using liquid chromatography – mass spectrometry has been described. ^{13}C labeled internal standards have been used in this study to ensure the results trueness. Mass spectrometer ionization performance and the ion optics operating conditions have been optimized using native fomonisin standard mixtures. Nonbounded fumonisins have been extracted from the analyzed samples with a water:methanol:acetonitrile mixture and the extracts were subsequently cleaned using a molecularly imprinted polymeric (MIP) solid phase extraction cartridges. Purified extracts were analyzed by LC/MS. The concentration of bounded fumonisins has been assessed indirectly by determination of the hydrolyzed fumonisin (HFB1, HFB2 and HFB3) contents formed after sample digestion in 2M KOH. Analytes were extracted with dichloromethane, evaporated, re-dissolved in a water:methanol and acetic acid mixture and analyzed. The HFBs content was calculated as a simple difference between the total fumonisins concentration (calculated after alkaline digestion) and the determined free fumonisins concentration. The validation experiments were performed using laboratory-made corn samples fortified with known amount of fumonisin standards. The corn used for experiments had the fumonisins content below the limits of quantification. In addition to assess the method trueness the analysis of the corn certified reference material has been included in the study. The application of the labeled fumonisin internal standards allowed to obtain good quality of results. For the free fumonisins the recovery rates were in 96–108% range with relative standard deviation in 2–8% range, in respect to the fortification level, while for the hydrolyzed fumonisins (HFBs), recovery and relative standard deviation amounted respectively 89–106% and 4–11%. Limits of quantification (LOQ) of the method were 10 µg kg-1 and 20 µg kg^{-1}, respectively for FB1/FB2/FB3 and HFB1/ HFB2/HFB3. Satisfactory results of the free fumonisin contents for the analyzed certified reference material sample were achieved. Ho wever, the sum of determined concentrations of fumonisins in the same reference material was much higher and statistically significantly different. This confirms the presence of the bounded fumonisin forms in this sample.

Keywords: *Free fumonisins, bounded fumonisins, Molecularly Imprinted Polymer (MIP), liquid chromatography /ion trap mass spectrometry*

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J-32
MATRIX REFERENCE MATERIALS FOR FUMONISIN B1 AND B2 IN MAIZE FLOUR

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Fumonisins belong to a quite wide spectrum of mycotoxins commonly reported as Fusarium-toxins. Produced mostly by *Fusarium verticillioides* and *Fusarium proliferatum*, they mainly occur in maize and maize based products. Several countries have established restrictions in foodstuffs for the sum of fumonisins B_1 and B_2. In the European Union, Regulation (EC) 1126/2007 sets maximum allowable levels as 4000 µg/kg in unprocessed maize, 1000 µg/kg in maize for human consumption and 200 µg/kg in maize based food for infants and young children. Matrix Reference Materials (M-RMs) and Certified Reference Materials (M-CRMs) are a key tool for laboratories in method development and validation, providing a basis for accuracy and precision assessment as well as for the study of other statistical parameters such as repeatability, reproducibility, linear range, limit of quantification and franrobustness. Two matrix reference materials for fumonisins were produced according to the ISO 35 Guide that provides approaches for the certification of M-RMs, including homogeneity study, stability study and inter-comparative study as a whole processes. Naturally contamined maize was ground with a centrifugal mill, homogenized, dispensed into foil-laminated pouches, heat sealed and subjected to β-irradiation. Homogeneity was assessed. Ten randomly selected pouches were tested in duplicate for the fumonisin B_1 and B_2 content with a HPLC/MS/MS method under ISO/IEC 17025 accreditation. Results were submitted to ANOVA analysis. No difference in the within-pouches and between-pouches variances were detected by the F-test at the 95% confidence level. A short time stability study with isochronous method demonstrated that materials were stable at the transport and storage temperatures. Data points were plotted and the regression lines were calculated. Slopes were tested for significance using a t-test. No significant slope at 95% confidence level was found. Ten laboratories from eight countries participated to the characterization study. Some of them were EU National Reference Laboratories, mostly performing this analysis under ISO/IEC 17025 accreditation. High-Performance Liquid-Chromatography (HPLC) followed by fluorescence or mass selective detection (MS) methods were applied. Four independent measurements had to be performed for each material. For each set of results, the mean value was calculated as the arithmetic mean of the individual measurements. The assigned value of MA111 (1169 ± 119 µg/kg for fumonisin B1 and 322 ± 36 µg/kg for fumonisin B_2) is the arithmetic means of means using the set of results accepted after statistical evaluation. Associated uncertainty components were calculated according to the EURACHEM/CITAC Guide. MA110 was intended to be approximate as a blank material. It was not possible to assign a concentration value because of the low level of contamination, therefore a value below a maximum level was assigned (< 50 µg/kg for both fumonisins).

Keywords: *Fumonisin, maize, Reference Materials*

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J-33
THE IMPACT OF THE BAKING PROCESS ON THE CONCENTRATION OF FREE AND BOUNDED FUMONISINS B1, B2 & B3 IN GLUTEN FREE BREAD

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Maize based products considered as food are of major importance all over the world. Corn is a basic component of the diet on the several continents and for the people that suffer from the gluten intolerance disorders. Fumonisins, produced mainly by the Fusarium sp. fungi are considered as possibly carcinogenic to humans. Fumonisins are commonly detected in corn grain and corn based products. In addition various technological operations applied during grain processing are not able to eliminate their residues completely. In the recent years it was discovered that fumonisins might occur in food products in bounded forms and conjugates with food macro components like proteins or carbohydrates. The latter might be formed during food processing but may also appear in the non-processed raw grain. The aim of this work was to assess the influence of the preparation method of the dough made of dry milled corn flour and the baking process on the contents of free and bounded fumonisins under the model conditions. Corn flour was produced from the grains naturally contaminated with fumonisins. Pastries were made from dough prepared with direct method using yeasts or with indirect method based on application of sourdough. Both free and hydrolyzed fumonisins were determined using liquid chromatography /ion trap mass spectrometry based method. Free fumonisins were extracted using a water:methanol:acetonitrile mixture and then purified using molecularly imprinted polymeric solid phase extraction cartridges. To calculate the concentration of bounded fumonisins, alkaline hydrolysis of free and bounded fumonisins was conducted in 2M KOH, then the hydrolyzed fumonisins were extracted with dichloromethane. Difference between the calculated total fumonisin content and the determined free fumonisin concentration was considered as the concentration of the bounded forms. The average concentration of free fumonisins (FB1+FB2+FB3) in the corn flour was 5101±561 μg kg^{-1} while the average total calculated concentration was approximately 8756±876 μg kg^{-1}. Ratio of the total fumonisins concentration to the free forms was 0.72. There was no statistically significant difference in concentration of free and total fumonisins found in bread made from dough prepared with different methods. The bread baking process significantly affected the fumonisins concentration. The total fumonisins content was reduced by 23–25% while the free fumonisins were reduced by 30–32% depending on the type of the baking method applied. After the baking process an increase in the bounded to free fumonisins ratio up to 0.83–0.95 was noticed.

Keywords: *Free fumonisins, bounded fumonisins, gluten free bread, liquid chromatography /ion trap mass spectrometry*

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J-34
DEVELOPMENT OF BIOCHIP BASED IMMUNOASSAYS FOR SIMULTANEOUS SCREENING OF ENDOPHYTE ALKALOIDS IN GRASS SEED

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Introduction. Ergot alkaloids are mycotoxins produced by fungi of all species of the Claviceps genus which parasitize the seed heads of living plants at the time of flowering. The fungus replaces the developing grain or seed with the alkaloid wintering body, known as ergot, ergot body or sclerotium. The sclerotia are harvested together with the cereals or grass and can thus lead to contamination of cereal-based food and feed products with ergot alkaloids, the ingestion of which can cause ergotism in humans and animals. Toxicosis in livestock grazed on endophyte-infected grasses have a pronounced negative economic effect on animal production. According to the Official Journal of the European Union, member states should monitor the presence of ergot alkaloids in cereals and cereal products intended for human consumption or animal feeding, The European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) has recently recommended restricting the use of medicines containing ergot derivatives.
This study aimed to develop biochip based immunoassays for the multi-analytical detection of a range of endophyte alkaloids.
Methods. Simultaneous competitive chemiluminescent immunoassays were employed. The capture in-house made polyclonal antibodies for the detection of alkaloid toxins were immobilised and stabilised on the biochip surface defining discrete test sites. The assays were applied to the Evidence Investigator analyser, which incorporates dedicated software to process and archive the multiple data generated. Samples were extracted from grass seeds by liquid/liquid extraction and only 50 µl of the test sample was required for the multi-analytical assessment.
Results. Initial analytical evaluation showed analyte 1: ergot alkaloids (generic) exhibits an assay range of 0–10 ppb with a typical half maximal inhibitory concentration (IC50) value of 0.4ppb (ergotamine equivalence) and has shown to detect all six predominantly present ergot alkaloids, i.e. ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their related –inines required by the EU. Cross-reactivity values of the predominant ergot alkaloids range from 10% to 240%. Analyte 2: Paxilline exhibits an assay range of 0–50 ppb with a typical IC_{50} value of 0.3 ppb. Initial studies with authentic grass seed samples indicate Limits of Detection (LOD) of <5ppb and <2ppb for ergot alkaloids (generic) and paxilline respectively. The intra-assay precision of the tests was typically <10%.
Conclusions. The results from this initial evaluation indicate applicability of biochip array technology to the screening of endophyte alkaloids in grass seed, which represents a fast and reliable method for the simultaneous detection of ergot alkaloids including ergotamine, ergometrine, ergosine, ergocristine, ergocryptine and ergocornine together with their corresponding -inine forms and paxilline from a single, low volume test sample.

Keywords: *Mycotoxins, ergot alkaloids, biochip array, grass seed, immunoassay*

J-35
DEVELOPMENT AND IN-HOUSE VALIDATION OF AN UHPLC–HR–ORBITRAP MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF LIPOPHILIC MARINE TOXINS IN SHELLFISH

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Up to now, analytical methods used for the detection of marine toxins have generally been focussing on the analysis of 13 lipophilic toxins. However, to date more than 200 lipophilic marine toxins have been described. During the last years, many analytical methods based on liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) have been consolidated by inter-laboratory validations. However, the main drawback of LC–MS/MS methods remains the limited number of compounds that can be analysed in a single run. Therefore, in this study, a method based on ultra high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry was developed and validated according to CD 2002/657/EC for confirmatory analysis of regulated lipophilic marine toxins in shellfish flesh. Development and validation of the analytical method was realized by consideration of 6 different marine lipophilic toxins, including okadaic acid (OA), dinophysistox in-1 (DTX-1), pectenotoxin-2 (PTX-2), azaspiracid-1 (AZA-1), yessotoxin (YTX) and 13-desmethyl spirolide C (SPX-1) The separation of the compounds was achieved using the reversed phase Nucleodur Gravity C18 (1.8 µm, 50 × 2 mm) column under alkaline conditions (using water and acetonitrile, both containing 0.01% ammonium hydroxide). Ionization of the analytes was carried out using a heated electrospray ionization probe (HESI-II), operating in positive and negative ion mode. Mass spectrometric analysis was executed at an *m/z* scan range of 100–1200 and a mass resolution of 50,000 full width at half maximum (FWHM). For the validation, six replicates at each of three levels of enrichment (0.5, 1 and 1.5 times the permitted limit) were analyzed on three consecutive days (day 1 and 2: mussel, day 3: oyster), since preliminary interspecies variation experiments proved to be below 4.9%. Quantification of the targeted marine toxins was realized by using matrix matched calibration cur ves. The obtained results for recovery, repeatability, within-laboratory reproducibility (RSDR), decision limit (CCα), linearity and ruggedness were compliant with CD recommendations. The linearity was evaluated by preparing 8-point calibration curves in matrix and correlation coefficients (R2) obtained for each compound were ≥ 0.99. For all compounds the RSDR ranged from 2.9 to 4.9%, repeatability from 2.9 to 4.8% and recovery from 90% to 112% for the three levels of enrichment. In addition, identification of the compounds was performed by consideration of the accurate m/z and confirmed by the presence of a second diagnostic ion (i.e. 13C isotopic ion). In conclusion, UHPLC–HR–Orbitrap MS permitted more accurate and faster detection of the targeted toxins than previously described LC–MS/MS methods. Furthermore, HRMS allows to retrospectively screen for many toxins analogues and metabolites using its full scan capabilities but also untargeted screening through the use of metabolomics software.

Keywords: *UHPLC–Orbitrap–HRMS; lipophilic marine toxins; Okadaic acid; CD 2002/657/EC*

J-36
FATE OF MYCOTOXINS IN THE CORNMEAL PROCESSING FROM CARYOPSYS TO PRE-COOKED PORRIDGE

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Corn and maize are major and important crops consumed as food and feed. Mycotoxins, mainly fumonisins and aflatoxins, are a crucial problem concerning food products based on maize grain. Fumonisins are secondary metabolites produced by fungi of the Fusarium genius, particularly by F. verticillioides and F. proliferatum. Fumonisins may cause a variety of diseases in animals, as well as hepatocarcinogenic, hepatotoxic, nephrotoxic and cytotoxic effect in mammals. Moreover, there is evidence of a high incidence of human esophageal cancer associated with FB1 exposure [1, 2]. The problem of fumonisin contamination is further complicated by the fact that hydrolyzed forms and degradation products were found in thermally treated products but also in mild-treated products, at the same time possible modifications of mycotoxin structure by interaction with other food components (masked forms) may take place [3, 4]. The main objective of this study was to improve the comprehension of fum onisin masking mechanism and the influence of different treatment on fumonisin and aflatoxin occurrence in samples collected from a cornmeal industrial production plant: pre-cleaned and cleaned corn, pre-cooked and cooked broken corn, pre-cooled and cooled corn-flake, pre-cooked flour “fumetto”, pre-cooked porridge, germ and middlings. Mycotoxins content was determined by HPLC–MS/MS analysis combined with adequate protocols for their extraction and clean-up. Results showed that no masked fumonisins were detected; higher amounts of contamination were detected in middlings (probably due to the presence of pericarp), while pre-cooked porridge was lower than the other fractions. All the analyzed samples intended for human consumption showed levels of contamination below the legal limits reported in EC Regulation 1126/2007; on the other hand, findings obtained for byproducts should be taken into consideration to assess feed safety and quality.

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Keywords: *Masked mycotoxins, cornmeal production, Fusarium, fumonisin*

J-37
THE USE OF MICROFLOW UHPLC IN MYCOTOXIN ANALYSIS

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Traditionally in mycotoxin screening of food samples, samples are extracted and analysed by LC/MS/MS usually at LC flow rates which are in excess of 500 µl/min and in combination with high pressures with smaller particle size HPLC columns to maintain sharp peaks and fast chromatography. These flow rates produce fast speeds and excellent peak shapes and results, but have a draw back in that they require higher volumes of organic solvent. The consumption of HPLC organic solvents, such as acetonitrile and methanol, is a growing cost of analysis and its disposal has an environmental impact. Therefore, ways to reduce solvent consumption in pesticide residue testing will be beneficial to the environment and reduce running costs of a testing lab. Here we present new data using microflow LC, running below 40 µL/min, in combination with a LC-MS/MS method developed on an AB SCIEX QTRAP® system utilizing the Scheduled MRM™ algorithm with the acquisition of MS/MS spectra for compound identification. Initially this approach has been applied to a screen of ergot alkaloids and alfatoxins to show its applicability in food analysis and data presented with compare Micro LC with traditional LC flow rates.

Keywords: *Mycotoxin Screening, Micro LC, fast speed analysis, reduced cost*

J-38
THE USE OF ULTRA-PERFORMANCE CONVERGENCE CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY (UPC2–MS/MS) IN MYCOTOXIN ANALYSIS

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The ultra-performance convergence chromatograph (UPC2) is a robust and reproducible supercritical fluid chromatograph (SFC) recently introduced by Waters (Milford, MA, USA) and able to work with sub 2 µm particles. A supercritical fluid is a substance at a temperature and pressure above its critical point, resulting in a substance which is neither a liquid nor a gas. In practice supercritical CO_2 is most often used as mobile phase to separate the analytes of interest. As a supercritical fluid CO_2 has a low viscosity allowing the use of higher flow rates which results in faster analysis or the use of longer columns resulting in an increased resolution. By combining SFC with sub 2 µm particles the analysis time or resolution can even be further improved. Moreover, according to the manufacturer UPC2 has a selectivity which is orthogonal to both reversed and normal phase HPLC, making this technique complementary to HPLC/UPLC. Furthermore, by using CO_2 as the liquid phase in chromatography the amount of used organic solvents can be reduced, making UPC2 a green chromatographic technique. Due to the very different physico-chemical properties of mycotoxins it has proven very challenging to determine different mycotoxins simultaneously. Although the most problems in the development of such a multi-mycotoxin method are situated in the extraction and clean-up steps, the use of HPLC/UPLC has also sometimes shown its limitations. For instance Monbaliu et al. (2009) showed that the separation of the isomers 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) (type-B trichothecene produced by Fusarium species) using HPLC was impossible and that the differentiation between these two compounds can only be made based on the detected ion ratios. This however can be very difficult depending on the analysed matrix. This poster will focus on the possibility and the added value of using UPC2–MS/MS for the first time in the analysis of mycotoxins.

Keywords: *Mycotoxins, UPC2–MS/MS*

J-39
BROAD SCREENING OF PHYCOTOXINS IN TISSUE, WATER AND FOOD SUPPLEMENTS WITH USE OF LIQUID CHROMATOGRAPHY FULL SCAN HIGH RESOLUTION MASS SPECTROMETRY

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Phycotoxins such as azaspiracids, microcystins, saxitoxins and ciguatoxins are produced by algae which are naturally occurring in marine and fresh water. Different types of phycotoxins can end up in matrices like shellfish, water or food supplements. Analytical methods are available to analyse regulated phycotoxins, however these methods are only suitable for a small specific group of toxins and/or a specific matrix (mainly shellfish). Therefore, a screening method is developed for all kinds of phycotoxins in different matrices such as shellfish, fresh and sea water and food supplements. To analyse lipophilic phycotoxins, water samples are cleaned and toxins are concentrated with use of solid phase extraction (SPE). Due to the chemical properties of hydrophilic phycotoxins, these toxins are not retained on a C18 SPE cartridge. Therefor the hydrophilic phycotoxins in water samples need to be concentrated by evaporation of the water. For shellfish samples and food supplements a different approach is needed. To extract the phycotoxins out of tissue (shellfish) and food supplements a two-step extraction is used. First methanol is added (lipophilic phycotoxins) followed by extraction using an ultrasonic disruptor. Second, a mixture of acetonitrile, water, ammonium formate and formic acid is used (hydrophilic phycotoxins) and vortex mixing. Both extracts are combined. Subsequently, food supplement extracts are cleaned with use of C18 SPE for lipophilic phycotoxins. Shellfish extracts are analysed with LC–hrMS directly after extraction. For separation, liquid chromatography methods are developed for lipophilic and hydrophilic phycotoxins. Lipophilic phycotoxins are separated by reversed phased chromatography (UPLC C18 BEH) as opposed to the hydrophilic phycotoxins, which are separated with use of a HILIC LC-column (TSKgel Amide-80). For both methods a gradient with a runtime of 20 minutes is used with the same mobile phase composition, water, acetonitrile, ammonium formate and formic acid. Subsequently the samples are measured with use of a full scan high resolution mass spectrometer (Orbitrap) at a resolution of 50.000 and in combination with HCD fragmentation. For the developed extraction procedures acceptable recoveries for the known phycotoxins are obtained between 70% and 150%. Compared to current available methods toxins are well retained on the LC-column. By using an in house created library and developed software (MetAlign) we were able to screen various sample types for over 300 different phycotoxins.

Keywords: *High resolution mass spectrometry, phycotoxins, marine biotoxins*

J-40
UPLC–MS/MS METHOD FOR THE ROUTINE QUANTIFICATION OF REGULATED AND NON-REGULATED LIPOPHILIC MARINE BIOTOXINS IN SHELLFISH

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Consumption of shellfish (mussels, oysters, clams, etc) contaminated with biotoxins can cause severe intoxications in humans such as Diarrhetic Shellfish Poisoning (DSP). Due to their lipophilic properties DSP toxins are often classified as lipophilic marine biotoxins. Marine biotoxins are naturally produced by different types of phytoplankton and are therefore also named phycotoxins. The complexity of the lipophilic marine biotoxins lies in the variety of physiochemical properties such as carboxylic acids, sulfonic acids, amino and imino functionalities. In the European Union (EU) legislation various toxin groups are regulated and these toxins should be monitored in official control programs. Before July 2011, the official method was a bioassay based on the oral administration of shellfish meat to a rat or the interperitoneal injection of a shellfish extract in mice. Since July 2011, the official method for control of shellfish on the presence of lipophilic marine biotoxins has been LC–MS/MS. The EU reference LC–MS/MS method is based on a fixed extraction procedure followed by separation using conventional LC separation with either an acidic mobile phase or alkaline mobile phase and detection by tandem quadrupole MS. The aims are to produce a much faster routine analysis than the conventional LC method under alkaline conditions, and to include additional non-regulated compounds that are of interest to EFSA. Analysing homogenized whole flesh shellfish tissue, many different classes of the regulated lipophilic marine biotoxins; and also some of the non-regulated cyclic imines, were analysed in a five-minute analysis using UPLC coupled to tandem quadrupole MS. The UPLC separation provided good results for all different toxins classes; only peak shapes for azaspiracids were somewhat negatively affected by the alkaline conditions but they could still be integrated sufficiently for routine analysis and date from the to single-day validation is shown. Sensitivity from the experiments was good: even at levels of 0.125 times the validation level (regulatory level for regulated toxins) a signal-to-noise above 3 could be obtained.

Keywords: *Marine biotoxins uplc MSMS*

J-41
TRACE LEVEL DETECTION OF MYCOTOXIN CONTAMINATION IN ANIMAL FEEDINGSTUFFS

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There are now over 400 recognised mycotoxins found in foods and animal feedings materials and it has been reported that as much as 25% of the world's cereal grains may be contaminated with mycotoxins. The majority of mycotoxins are extremely stable and not destroyed by food processing or cooking and as such can easily enter the marketplace. At present, mycotoxin contamination represents the highest number of notifications under the EU Rapid Alert System for Food and Feeds (RASFF). The analysis of animal feeds including silage represents a major analytical challenge due to the complexity and in-homogeneity of these matrices. Although permitted limits for mycotoxins are set at relatively high (mg kg^{-1}) concentrations in the EU, toxic effects, e.g. immunotoxicity and feed uptake problems in certain species (poultry and porcines) are often observed at low (µg kg^{-1}) concentrations. For this reason there is often an economic requirement to achieve low detection limits in this matrix. There is also a high potential for co-contamination in this matrix (due to pre and post harvest infestation) resulting in the occurrence of tricothecenes, aflatoxins, fumonisins, ochratoxin, patulin, T-2/HT-2 and alternaria toxins for example within a single sample. Within this paper we report the development of a quantitative method for the determination of circa 35 relevant mycotoxins in a variety of animal feed and silage extracts. A generic and simplified sample extraction protocol based on 84:16 (*v/v*) acetonitrile: acidified water for the recovery of mycotoxins was used, and analysed using an ACQUITY UPLC and Xevo TQ-S tandem quadrupole mass spectrometer. We investigate the affect of matrix dilution coupled to high instrument sensitivity to overcome common analytical challenges such as ion suppression. We also exploit the functionality of the Xevo TQ-S: to monitor background interference profile simultaneously during MRM transitions; the use of product ion confirmation to provide additional confidence in identifications; and Quanpedia for automated MRM scheduling and method development.

Keywords: *Mycotoxins; LC–MS/MS; animal feeds; silage*

J-42
OCCURRENCE OF AFLATOXIN M1 IN MILK IN THAILAND

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The contamination of aflatoxinM1 in raw milk and pasteurized milk is the result of aflatoxinB1 contamination on feedstuffs. AflatoxinM1 is less toxic than aflatoxinB1, however, this mycotoxin is a source of concern for the infant food industry and it is a serious problem for public health. So, the aim of this study was to determine the occurrence and the level of aflatoxin M1 in raw milk and pasteurized milk in Thailand. Milk samples were randomly taken from local farms and from markets. They were examined for aflatoxin M1 by using immuno-affinity column (IAC) and High Performance Liquid Chromatography (HPLC). The result shown that aflatoxinM1 was found 64% in milk samples (32/50, average 0.059 ppb), 61.9% in raw milk (13/21, average 0.067ppb), 65.51% in pasteurized milk (19/29, average 0.053ppb). The method was employed to analyze aflatoxin M1 and resulted % recovery was 84% of 0.1ppb and 65.34% of 0.2 ppb. Our results showed that the concentration of aflatoxin M1 did not exceed the limit of USA (0.5ppb).

Keywords: *Aflatoxin M1, milk*

Acknowledgement: *Thammasat University, Thailand*

J-43
OCCURRENCE OF AFLATOXIN B1 IN FOOD PRODUCTS IN THAILAND

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Aflatoxin B1 is a mycotoxin which possesses a variety of toxic effects. It is produced by fungi for which food stuffs such as beans, cereals, fruits and seeds. Many kinds of food and feed can be contaminated with mycotoxins since they can be formed in commodities before and after harvest. It has proven itself at least partly resistant to food processing methods meaning it is also present in derived products. So, the aim of this study was to determine the possibility of contamination of aflatoxinB1 in food products in Thailand. The 70 food samples were purchased from markets which around in Bangkok. They were divided into 4 categories, 5 samples of imported blue cheese, 7 samples of local fermented alcoholic, 18 samples of fermented soybean products and 40 samples of peanut products. They were determined for aflatoxin B1 by ELISA method. The revealed rated of aflatoxinB1 contamination were 71.42%, 100%, 83.33% and 90% for the alcoholic, blue cheese, soybean and peanut samples, respectively. The individual values, with each category samples, range from 0.3 to 2.15 ppb(average 0.48 ppb), 0.5–1.25 ppb(average0.95 ppb), 0.2–3.2 ppb(average 1.54 ppb) and 0.1–73.85 ppb(average 5.6 ppb) for alcoholic, blue cheese, soybean and peanut samples, respectively. This work represents the data of the first survey on the occurrence of aflatoxinB1 in various food products in Thailand. So, the confirmation work should be done in the next work, not only ELISA method but also including with immunoaffinity cleaning column and HPLC as well as increasing the number of sample size.

Keywords: *Mycotoxins; AflatoxinB1*

Acknowledgement: *Thammasat University, Thailand*

J-44
OCCURRENCE OF MOULDS AND AFLATOXIN IN PEANUT AND CEREAL PRODUCTS FROM WHOLE SALE MARKET IN THAILAND

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Peanut and cereal products were collected from market in Pathum Thani, Thailand. They were examined for the presence of food-borne fungi and analyzed for aflatoxin (AFs) using HPLC equipped with florescence detector. In total plate and fungal counts technique, the plate and fungal counts in peanuts sample were 3.5×10^3 cfu/g and 4.5×10^2cfu/g, respectively. While the cereal plate and fungal counts were 7.6×10^2 cfu/g and 1.3×10^2 cfu/g. Three fungal genus in the samples were isolated and identified as *Aspergillus* spp., *Penicillum* spp. and *Trichoderma* spp. based on its growth characteristics in different media and morphology.*Aspergillus* spp. was accounted for 13.7% the most frequent genus, while *Penicillum* spp. and *Trichoderma* spp. were 9.8% and 1.96%. Aflatoxin B_1 was detected in roasted peanuts (20%) and peanut products (10%). The average of aflatoxin B_1 level was 4.01 µg/kg in roasted peanuts and 7.99 µg/kg in peanut products. The aflatoxin B_2 detected in roasted peanut was 0.22 µg/kg and 1.98 µg/kg in peanut products. Aflatoxins B_1 and aflatoxins B_2 were not found in cereal products.

Keyword moulds, *aflatoxin, peanut, cereal products*

J-45
DEVELOPMENT OF A MULTI-MYCOTOXIN METHOD USING UPLC–MS/MS AND ITS APPLICATION TO ANALYSIS OF ANIMAL FEED MATERIALS

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Within the framework of the EU funded project QSAFFE (Quality and Safety of Food and Feed in Europe) a new method for the simultaneous analysis of >50 mycotoxins was developed. A simple extraction protocol that used acetonitrile:water: acetic acid was used. No clean-up was used to in an effort to retain as many of the analytes as possible. Samples were analysed using a Waters UPLC system with a XEVO TQS mass spectrometer. Two analytical runs, one using neutral mobile phase conditions and one using acidic conditions, were required to ensure optimum chromatographic performance and ionisation of analytes. The method was capable of detecting several groups of mycotoxins including aflatoxins, fumonisins, trichothecenes, Alternaria toxins, ergot alkaloids, zearalenone and derivatives, enniatins, as well as many other Fusarium and Penicillium mycotoxins. In addition masked forms of some of the mycotoxins were also included. The method was applied to the analysis of ~150 animal feed materials that were sourced as part of the QSAFFE project. Samples were from different geographical origins, and covered several main groups including maize, soy, rape, sunflower and Dried Distillers Grains with Solubles (DDGS). The method was also applied to the analysis of wheat that had been inoculated with Fusarium in the field. High levels of deoxynivalenol, deoxynivalenol- 3-glucoside, nivalenol, zearalenone and it derivatives, and moniliformin were found in the samples as well as extremely high levels of enniatins. Selected contaminated samples were used in fermentation experiments to model the fate of these mycotoxins in laboratory experiments designed to represent conditions used in bioethanol and DDGS production processes. The results were used to carry out mass balance calculations to determine effect of the process on mycotoxin concentrations.

Keywords: *mycotoxins, feed, UPLC–MS/MS, fate*

Acknowledgement: *Alison Lowham, Patrick Hogrel*

J-46
ANALYSIS OF MYCOTOXINS IN FOOD MATRICES USING QUECHERS AND LC–MS/MS

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Because of the significant health risk to both humans and livestock, mycotoxin monitoring is extremely important for effective food regulation. Mycotoxin monitoring is conducted by laboratories and typically involves extraction, cleanup and HPLC analysis. The presented method for mycotoxin analysis utilizes extraction and cleanup with Quechers followed by two HPLC analytical methods. The first method is used for routine screening. It has a fast run time but doesn't fully resolve 3-Acetyldeoxynivalenol and 15-Acetyldeoxynivalenol. The second method is a confirmatory method that resolves all compounds allowing for highly accurate quantitation in case of a positive screening result. The analytical methods were tested on 3 different food matrices containing various amounts of pigmentation. Raspberries were used to represent highly pigmented foods, corn was used to represent a medium level of pigmentation and rice crispy cereal was used to represent a low level of pigmentation. Excellent accuracy and fast analysis times were achieved using this Quechers and LC–MS/MS analytical approach.

Keywords: *Mycotoxin, Quechers, ROQ, LC–MSMS*

J-47
NOVEL, RAPID, SENSITIVE, FLUORESCENT PLANAR WAVEGUIDE IMMUNOASSAYS FOR THE DETECTION OF PARALYTIC SHELLFISH TOXINS IN MARINE ALGAE AND MICROCYSTINS IN FRESHWATER AND CYANOBACTERIAL EXTRACTS.

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Alexandrium is an armoured dinoflagellate found in marine waters that produces paralytic shellfish poisoning (PSP) toxins. These toxins can enter the food chain through accumulation in shellfish and pose a serious risk to human and animal health, causing rapid paralysis and death. Cyanobacteria are widespread throughout global waters and of the many types, Microcystis aeruginosa (predominantly freshwater) is the most common species. The cyanobacterium produces toxins known as microcystins (MCs) that can contaminate public water supplies and natural water bodies and have been responsible for poisonings and deaths in humans and animals. Excessive growth of these organisms due to climate change and increasing pollution pose an increasing threat across the globe in terms of health risks and economic burden and therefore it is necessary to have the ability to detect these biotoxins with low cost, high quality and rapid diagnostic tests. Through the US-Ireland Partnership Program, tests for the detection of these toxins were developed using novel sensor technology produced by mBio Diagnostics Inc., Boulder, Colorado, USA. Innovative sample preparation methods were exploited to ensure analysis was completed within a short period of time and to enable use in both laboratory and field environments. Monitoring of coastal waters for toxins produced by marine dinoflagellates is of paramount importance to provide an early warning of risk. In this study a rapid, easy-to-use, portable fluorescent planar waveguide immunoassay has been developed and validated to meet these requirements. In conjunction with a novel sample preparation, the total time required to test a sample is approximately 20 minutes. The assay was validated and the LOD and CCβ were determined as 12 pg/mL and 20 pg/mL respectively with an intra-assay CV of 11.3% at the CCβ and an average recovery of 106%. The highly innovative assay was proven to accurately detect toxin presence in algae sampled from the US and European waters at an unprecedented cell density of 10cells/L. The microcystin assay was developed, and validated allowing the determination of the total microcystin content in a sample, i.e. both free and cell-bound levels. The planar waveguide assay is rapid, taking only 15 min and detects the more common, and toxic, variants of microcystin. The LOD and CCβ were determined as 0.78 ng/ml and 1 ng/ml respectively. Intra-assay and inter-assay analyses displayed CVs between 5% and 26% with recoveries ranging from 73% to 101%. Comparison with LC–MS/MS showed a high correlation (R^2=0.995) between the calculated concentrations of 5 Microcystis aeruginosa cultures for total microcystin content. The assay can detect free microcystins to 1 ng/mL and cell bound microcystins to 0.1 ng/mL. This new tool will detect harmful toxins with much greater accuracy and lower cost, helping to safeguard the shellfish, water and tourism industries, and to protect public health.

Keywords: *Paralytic shellfish toxins, microcystins, fluorescence immunoassay, planar waveguide, sensitive.*

J-48
SINGLE LABORATORY VALIDATION OF AN EXTENSION OF THE PRECOLUMN OXIDATION HPLC FLUORESCENCE DETECTION METHOD FOR THE ANALYSIS OF PARALYTIC SHELLFISH POISONING TOXINS

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A pre-column oxidation HPLC method with fluorescence detection was internationally validated through a collaborative trial in 2005 and subsequently adopted as AOAC 2005.06 Official Method for the Analysis of Paralytic Shellfish Toxins (PSTs). The availability of new PSTs standards as well as the appearance of new toxin profiles, such as the ones from *Gymnodinium catenatum*, made necessary the adaptation to the new situation. With this aim a single laboratory validation was designed for the implemented precolumn HPLC-FLD method. Evaluation of the performance characteristics for dc-GTX2,3, C1,2, dcSTX, GTX2,3, GTX5, STX, GTX1,4, NEO, dcNEO, GTX6 and C3,4 was conducted following IUPAC and Eurachem Guidelines. Preliminary studies involved the assessment of the potential effects of impurities present in different certified reference materials in the quantification of dc-GTX2,3, C1,2, dcSTX, GTX2,3, GTX5, STX, with the aim of avoiding separation of standards into two different calibration mixtures. Evaluation of method selectivity involved the determination of naturally fluorescent compounds which could potentially interfere with the PSTs determination and the investigation of possible matrix effects by spiking standards in different sample extracts. LODs and theoretical LOQs were calculated from PSTs S/N ratios of 3:1 and 10:1 respectively. Blank extracts were spiked in quintuplicate to establish the practical LOQs by evaluating recoveries and repeatability precision data. Linear range was evaluated in solvent standards from each toxin LOQ to a concentration of 1 or 2 µM (depending on the toxins standards availability). Accuracy (recovery evaluation and precision under repeatabilit y conditions) were also evaluated at the levels of 400 and 800 µg eq. STX.diHCl/kg, whenever possible. Data from precision under repeatability and intralaboratory reproducibility conditions were collected from the analysis of different naturally contaminated shellfish over the years (*Alexandrium spp.* and *Gymnodinium catenatum* toxic profiles). Traceability was evaluated through the analysis of the tissue certified reference material commercially available (PO PST MRC 1101). The evaluation of the robustness was focused on the assessment of the stability of either peroxide or periodate oxidised standards over time. Method uncertainty was evaluated for most toxins at the practical LOQs, 400 and 800 µg eq. STX.diHCl/kg. This work shows the results obtained on the single laboratory validation carried out at the EU Reference Laboratory for Marine Biotoxins.

Keywords: *PSP, HPLC, Pre-column oxidation, Validation*

J-49
DEVELOPMENT OF AN AUTOMATED SAMPLE PREPARATION AND ANALYSIS WORKFLOW FOR THE DETERMINATION OF MYCOTOXIN RESIDUES IN DIFFERENT FOOD MATRICES

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The abundance of mycotoxins in food products is a major concern in product safety due to the health risks they pose to humans and livestock. Mycotoxin levels in food and animal feed are regulated in most countries and there is great interest in a fast, sensitive, and selective analysis method. Determining mycotoxin concentrations at trace levels in the presence of large amounts of sample matrix is a challenging task, making the accuracy and precision of the analytical results fundamentally dependent on the sample preparation methodologies used to isolate the mycotoxins from the complex food and animal feed matrices. In this report, we describe an automated sample preparation and analysis workflow for the screening of multi-mycotoxin residues in different food matrices (corn, wheat, rice) by LC–MS/MS. The extraction methodology was performed using a GERSTEL MPS robotic autosampler interfaced to an AB SCIEX QTRAP® 4500 LC–MS/MS System. The automated sample cleanup and analysis workflow targeted a panel of 14 mycotoxins (aflatoxins, trichotecenes and fuminosins). The LC–MS/MS was operated in Multiple Reaction Mode (MRM) with fast polarity switching for accurate detection. Dependent MS/MS spectra were also acquired in the Enhanced Product Ion (EPI) mode after being triggered from a Scheduled MRM™ Information Dependent Acquisition (IDA) survey scan. The automated SPE cleanup procedure provided extraction efficiencies greater than 70% for all mycotoxins screened in the different food samples with RSDs less than 15%. In addition; good linearity was achieved (R^2 values of 0.98 or greater) reaching limits of quantitation lower than the action levels established by the FDA for most analytes. The ability to automate Solid Phase Extraction clean-up methodologies focused on mycotoxin sample extracts and to couple the extraction directly to LC–MS/MS allows compound identification with highest confidence based on mass spectral library matching, and improves laboratory productivity by streamlining the complete analytical process.

Keywords: *Mycotoxins, Automation, LC–MS/MS, Sample Preparation, Residue Screening*

J-50
ALTERNATIVE DERIVATIZATION PROTOCOLS FOR THE DETERMINATION OF FUMONISINS B1 AND B2 IN WINES BY HPLC–FLD

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Fumonisins (FMs) are a group of mycotoxins, produced mainly by *Fusarium verticillioides, F. proliferatum, Gibberella fujikuroi* and *Aspergillus niger*. They contaminate primarily the maize and other cereals (sorghum, rice, etc) but they are detected also in grapes and raisins and they are displayed in the must and wine. Fumonisins cause toxicity in the liver and kidney, immunosuppression and neurotoxicity. So far, at least 28 fumonisins have been isolated and the World Organization for Research on Cancer (IARC) has classified fumonisin B1 as potentially carcinogenic (category 2B). The aim of this study was to develop a methodology for determining fumonisin B1 and B2 (FB1 and FB2) in commercial wines, using two different protocols. In the first protocol, derivatization before column with the derivatization reagent, ortho-phthalaldehyde (OPA) was applied. The derivatization reagent was added to each standard solution of fumonisins before injection and analysis by high performanc e liquid chromatography, (HPLC) with fluorescence detector (FLD) was followed. In the second protocol, post-column derivatization of standard solutions of fumonisns, using the same derivatization reagent (OPA) was applied. The comparison of the two protocols was performed with the assess of linearity and sensitivity, in a concentration range of 50-2000 ng/mL. For each protocol of analysis, reference curves (one for each fumonisin) with 6 points were plotted. The correlation coefficients (r^2) for FB1 and FB2, were 0.984 and 0.948, respectively, for the first protocol, while for the second protocol the values of r^2 were 0.987 and 0.990 for FB1 and FB2, respectively. Also from the reference curves, the detection limits (LOD) and limits of quantification (LOQ) for both fumonisins were calculated. The detection limits for the first protocol were LOD FB1 = 120 ng/mL and LOD FB2 = 360 ng/mL and for the second were LODFB1 = 102 ng/mL and LOD FB2 = 306 ng/mL, while the limits of quantif ication for the first protocol were LOQ FB1 = 63 ng/mL and LOQ FB2 = 189 ng/mL and for the second were LOQ FB1 = 27 ng/mL and LOQ FB2 = 81 ng/mL. Finally, the second protocol analysis was chosen and applied for the determination of fumonisins FB1 and FB2 in 22 samples of commercial red wines from 8 countries.

Keywords: *Fumonisins; red wines; OPA; HPLC*

J-51
SIMULTANEOUS DETERMINATION OF TOTAL AFLATOXIN, OCHRATOXIN A AND FUMONISIN USING AOF MS–PREP® IN CONJUNCTION WITH LC–MS/MS

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European legislative limits for total aflatoxin, ochratoxin A and fumonisins are in place for cereals and cereal products. There is a demand in the market for faster and less labour intensive tests. Immunoaffinity columns are rapidly becoming the routine standard method of choice for complying with regulatory mycotoxin analysis however there is a growing need for multi-mycotoxin analysis using a single extraction method. In response, R-Biopharm Rhone has produced a multi-toxin immunoaffinity column, AOF MS-PREP® nabling the isolation and concentration of aflatoxins B1, B2, G1, G2, ochratoxin and fumonisin in cereals and cereal products. The advantages of this new immunoaffinity column are that only one sample preparation method and one single LC–MS/MS run is required for quantifying all four mycotoxins therefore having greater sample throughput and a reduction in the use of solvents and consumables.

Keywords: *aflatoxin, ochratoxin A, fumonisins, LC–MS/MS, Immunoaffinity*

J-52
LEVELS OF AFLATOXIN M1 IN MILK ON SERBIAN MARKET

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Since milk has an important role in human nutrition, especially in children's diet, its contamination with aflatoxin M1 might be considered as a potential risk for human health. The aim of this study was to determine the levels of aflatoxin M1 presence in milk samples on Serbian market. Aflatoxin M1 (AFM1) was determinated by high performance liquid chromatography using a fluorescent detector after cleanup of samples on immunoaffinity columns. The limit of quantification of method was 0.010 µg/kg. The recoveries were between 95.4 and 105.8%, and the RSDs in the range of 0.4 to 3.6%. This method was successfully applied for the of 111 milk samples. Aflatoxin M1 was detected in 95 (85.6%) of all samples. However, 62 (55.9%) samples among these was contaminated at a level above the maximum permissible limit accepted by the European Union legislation (50 ng/L) and 4 (0.04%) above the National level (500 ng/L) for aflatoxin M1.

Keywords: *aflatoxin M1, milk, HPLC, immunoaffinity columns*

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J-53
DEVELOPMENT OF APTAMERS FOR A RAPID DETECTION OF AFLATOXIN IN MAIZE

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Aflatoxins are mycotoxins produced by two species of the fungus Aspergillus, namely Aspergillus flavus and Aspergillus parasiticus. These species are widespread in nature and can colonize many important agricultural crops such as cereal grains and legumes. Aflatoxins are primarily found in warmer regions under moist conditions. Among several naturally occurring aflatoxins, the aflatoxin B1 is the most common one in food and also the most toxic from among the aflatoxins. Until now two strategies have been established for the detection and quantification of these mycotoxins: either analysis with high-end equipment such as HPLC–UV and LC–MS based methods or rapid screening protocols with immuno-assays such as ELISA and lateral flow devices. However, both approaches suffer from particular drawbacks: instrumental analysis require expensive equipment and highly skilled personnel and the immuno-tests rely on antibodies. The production and the purification of approp riate antibodies is expensive and elaborate. Furthermore, antibodies are not stable under higher ambient temperatures and hence their shelf-life is rather short. In the recent years aptamers have been considered as a potential alternative to antibodies. These single stranded DNA oligonucleotides have the ability to bind to a target molecule with similar binding constants as antibodies. Once the specific sequence of an aptamer is known it can be synthesized completely synthetically and the costs therefore are low. Aptamers are selected from a random library of oligonucleotides by a procedure called SELEX (Systematic Evolution of Ligands by EXponential enrichment). Here we describe the way towards aflatoxin binding single stranded aptamers. First the aflatoxin was bound to magnetic beads which were incubated with a random DNA library consisting of approximately 10^15 different sequences. These numerous sequences were subjected to the iterative selection SELEX procedure and the conditions were varied in each of the 13 rounds to artificially evolve aflatoxin binding DNA oligonucleotides. Until now no aptamer sequences for aflatoxins have been published.

Keywords: *Aptamer, aflatoxin, SELEX*

J-54
ANALYTICAL METHODS OF AFLATOXINS IN HERBAL MEDICINE AND HEALTH FUNCTIONAL FOODS

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Aflatoxins (AFs) are secondary metabolites of Aspergillus flavus and Aspergillus paraticus. They have high toxicity such as carcinogenic, teratogenic, and mutagenic properties. Analytical methods of aflatoxins (AFs: B1, B2, G1 and G2) in herbal medicines (HMs) and health functional foods (HFFs) were developed and analyzed in samples. For obtaining the representative samples, samples were directly collected from the users (total 2348 examinees) of HMs and HFFs. Samples were collected from national wide area except Jeju Island in South Korea. Subjects were 1015 adults (over 19 ages), 557 children and youths (7–18 ages), 488 infant and toddlers (0–6 ages) with 288 their parents. Two analytical methods such as trifluoroacetic acid and Kobra cell derivatization methods were compared. Kobra cell method had lower LOD than TFA method. Limit of detection of aflatoxin G2 in TFA and Kobra cell method were 2.28 and 0.07 ng g^{-1}, respectively. R-squared was closer to 1.00 in Kobra cell method. The baseline of the chromatogram of Kobra cell was stable. In addition, the resolution and sensitivity of Kobra cell method was better than that of TFA method. For the accurate analysis, Kobra cell method was selected for the further analysis in this study. Recovery rate of AFs using various matrixes such as solid, semi-solid, liquid samples and CRM was 81.81–119.87%. Z-score and linearities of calibration curves were 0.53 and 0.9996–0.9999, respectively. Total 241 samples (185 HFFs and 56 HMs) were analyzed by Kobra cell derivatization method. In this study, AFs were not detected in HFFs and HMs except Angelica gigas NAKAI extract products (HFF). Each contamination levels of aflatoxin G2 in the samples were 7.93 and 5.70 ng g^{-1}. Angelica gigas NAKAI is widely used as herb medicines. It is expected that production and refining process of HFFs scarcely affected AFs levels in HFF products. The levels of AFs in other HFF products were similar to t he previous studies reporting that AFs were not found in plant-derived dietary supplements and medicinal plants products. The importance of this study is, firstly, to develop much quicker, easier and economical method. Secondly, lots of different dietary supplements were analyzed.

Keywords: *Aflatoxin, Herbal medicine, Health functional foods, Immunoaffinity column, Kobra cell*

J-55
A NOVEL METHOD FOR RAPID DETERMINATION OF AFLATOXIN M1 IN MILK BY USING LC–MS/MS

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Background: Aflatoxins are a group of mycotoxins produced by certain fungi, especially *Aspergillus Flavus* and *A. Parasiticus*. There are four major naturally occurring aflatoxins: B1, B2, G1 and G2. Among them, aflatoxin B1 (AFB1) is notoriously the most common and toxic.. The International Agency for Research on Cancer of the World Health Organization (WHO) has classified AFB1 as a Group 1 human carcinogen. Aflatoxin M1 (AFM1) is the hydroxylated metabolite of AFB1. For the first time, AFM1 was found in the milk of lactating animals that consume feedstuffs contaminated with AFB1 in the 1960s. For aflatoxin M1, the Scientific Committee for Food concluded that there is sufficient evidences that aflatoxin notoxic is carcinogen. The European Community prescribes that the maximum residue level (MRL) of AFM1 in liquid milk should not exceed 0.05 $ngml^{-1}$. Such low MRL limits require highly sensitive methods for the detection and quantification of AFM1 in milk.
Aim of the study: The aim of our study has been to develop a simple and fast aflatoxin M1 by LC-MS/MS method for Milk and baby formulas.
Materials and methods: We have sourced milk from the local markets and we are using the Jasem 5 minute sample preparation method. Place 1.0 ml milk sample into a centrifuge tube – add 3.0 ml Reagent 1 onto sample and vortex – centrifuge at 2000 rpm for 3 minutes - filter supernatant into a HPLC vial and inject. We have used liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (LC–ESI–MS/MS, Agilent's Triple Quad MS 6460). The Run time takes only 7.5 minutes (run to run).
Results: Quantitative analysis is carried out by using multiple reaction monitoring (MRM) mode and run to run analysis time takes only 7.5 min. In contradiction to existing reference methods, this new method has no need to use an immunoaffinity column as a clean-up device – this provides lower analyzing cost and shorter sample preparation time with only 5 minutes. The calibration curve was linear between 0.006 ng/ml to 2 ng/ml.
Conclusion: This new method is a real breakthrough: very easy, fast, reliable, sensitive and low in cost for the routine monitoring of aflatoxin M1 in milk.

Keywords: *Food analysis, aflatoxin M1, milk*

J-56
NEW-FAST AND EASY METHOD FOR LC–MS/MS ANALYSIS OF PATULIN IN APPLE JUICE

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Background: Patulin is one of the mycotoxins which is found in apples and apple products. This mycotoxin is an important quality criteria for apple juice. Patulin is produced by certain genus of moulds, particularly Penicillium Expansum during processing owing to its solubility in water. Patulin has a mutagenic effects, it's carcinogenicity and teratogenicty have many times been reported. Because of those recognized adverse effects, food safety departments of many countries decided to control and monitor its level in apple juice and other food products made from apples. The maximum permitted concentration has been set for Patulin at 50 µg/L (ppb) in foodstuffs by the World Health Organisation (WHO). However, the present Reference Method (AOAC Official Method 2000.02 Patulin in Clear and Cloudy Apple Juices and Apple Puree) for Patulin is a Liquid Chromatographic-UV Method. This method takes very long with approximately 1 hour sample preparation time, is also highly complicated and offers only a very low sensitivity. It is applicable to determine of Patulin at ~ 25 ppb. Aim of the study: To develop an easy and fast method for the determination of Patulin with a very high sensitivity such as 0.2 to 0.5 ppb.
Materials and methods: Apple juice from the local market, LC–MS/MS based analysis developed with Agilent's Triple Quad MS 6460. No need sample preparation step for clear apple juice! Just inject into the system – only if necessary, filter apple juice before injection (cloudy juice).
Results: Jasem developed a Patulin LC–MS/MS method with which there is no need for sample prep such as Liquid-Liquid extraction or any concentration steps. It offers a 60x Higher Sample throughput: with as results an increased instrument productivity. Direct injection and very short and ultra-easy sample preparation in only 4–5 minutes, if sample is cloudy or it is a puree. The total run time is only 6 minutes. Te Jasem method is more selective and 40 times more sensitive than the standard HPLC–UV method, although no concentration and cleaning steps are used. Our method's sensitivity is 0.6 ppb LOQ (as a mentioned before, ref. method applicable of determination of Patulin is > 25 ppb).
Conclusion: With the Jasem Patulin LC-MS/MS commercial kit, it is possible to have a 60× higher sample throughput with very high sensitivity and – in most cases - without any sample prep.

Keywords: *LC–MS/MS, Analysis, Patulin, Apple Juice*

J-57

ARGENTINIAN & BRAZILIAN BOVINE MILK POWDER PRODUCING CHAIN PATHWAYS FOR AFLATOXIN M1 CONTAMINATION PREVENTION

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Argentina and Brazil are the largest milk producers in South America. Altogether, both countries are responsible for 7% of world milk production with 10,501,900 & 30,175,500 tons/year and an internal consumption of 201.9 & 150.0 L /person/year, respectively. Although Argentina production supplies its internal market, it can also export. On the other hand, Brazil market consumes its entire production and needs to import (mainly *milk powder*) from Argentina. Regarding *milk powder*, Argentina is the 4th producer in the world, after New Zealand, Europe, Australia and its main market for that product is Brazil followed by Venezuela. Despite this, Brazil exports some milk (*condensed milk*) for Africa (Angola & South Africa). Both countries main *milk* production regions are the same as the main *grain* (corn/wheat/rice/sorghum/soya beans) production. Dairy cattle farm ranges from small (10 cows) to mega (1000 cows) ones being the technology applied, both artisanal and advanced. Both countries dairy cattle feeding comprises, apart from pasture, also of pasture supplementation (depending on the time of the year and pasture conditions though). The supplements are: *silage* (maize & sorghum whole plant), *energy concentrate* (sorghum+corn+cotton seeds) and *industry by-products* (sunflower seed hulls, malt extract & extrused feed). In Brazil, by-products from the sugar cane and citrus industries are also utilized. As far as milk quality is concerned, either *composition* (protein and fat), *hygiene* (total bacteria count) or *sanitation* (somatic cells), as well as *diseases* control (brucellosis and tuberculosis), are monitored accordingly to each country´s Quality and Disease Eradication Official Programs. Both countries do not allow either chemical/natural contaminants (antibiotics/hormones/pesticides/mycotoxins) and water or other substances added to milk. Mycotoxins regulation are set for both dairy cattle feed and milk (*in natura* or *powder*). Regarding aflatoxins (AFLs) contamination, either in feed or in milk (AFM_1) that can pass into cow's milk fed with contaminated diet: there have been developed and applied Farm Guides and Trainings carried out by the agriculture authorities to prevent and control possible contamination. Although, some AFLs can be inactivated by the rumen flora, the remaining are transformed into AFM_1 (toxicity similar to AFB_1 & liver the target organ) and transferred to milk – *the main babies food* after breast feeding. Mercosur (South Cone common market, mainly Argentina/Brazil/Paraguay/Uruguay Countries) regulation establishes the: (a) maximum tolerance level – MTL for fluid and *powder milk* of 0.5 and 5.0 µg/L, respectively, (b) sampling (FIL-IDF50B) and (c) AFM_1 method (AOAC 980.21, 2005) considered appropriate for the purposes of the MTL set. Despite this, as other countries (such the European), are more restricted (MTL: 10 times lower), Mercosur countries have been discussing a reduction of AFM_1 so to decrease the infant population toxin exposure risk by milk ingestion and encourage South American dairy products exports to new / more MTL restricted markets. During the Argentina and Brazil Milk Food Chain Project, dairy cattle diet samples were collected, farms were evaluated and regulation/agricultural practices surveyed, in order to check its current status and changes recommended. *Milk powder* (skimmed, baby formula) from Argentina and Brazil were also randomly purchased from bouth countries supermarkets (sampling: European Union regulation - EC, 401/2006). The methodology applied was by liquid chromatography and fluoresce detection with immune-affinity column clean up and trifluoroacetic acid derivatization (LOD and LOQ: 0.03 and 0.1 µg/Kg, respectively). AFM_1 levels in *powder milk* varied from 0.10 to 0.92 and < LOQ to 0.81 µg/Kg for Argentina and Brazilian samples, respectively. None exceeded the Mercosur MTL (5 µg / kg). To control AFM_1 in food, it is necessary to reduce AFB_1 in feed for dairy cattle *by preventing fungi growth* and AFB_1 formation in agricultural commodities intended for animal use.

Keywords: *Milk Powder, Aflatoxin M1, Argentina, Brazil*

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J-58
PYRROLIZIDINE ALKALOIDS: TOXIC PLANT METABOLITES IN OUR DIET – TAKING ON THE ANALYTICAL CHALLENGE

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Pyrrolizidine alkaloids (PAs) are a large class of structurally diverse metabolites produced by many plants. Their main function is to protect plants from being consumed by insects and mammalian herbivores. In high doses PAs are hepatotoxic and exposure can result in hepatic veno-occlusive disease (HVOD), necrosis of the liver and ultimately death in humans and animals. Chronic, low level consumption of PAs may present a health risk as well, as the European Food Safety Authority (EFSA) has recently stated in their 2011 Scientific Opinion (EFSA J, 9:2406). PAs are regarded genotoxic carcinogens for which no Acceptable Daily Intake (ADI) could be established. Following a Margin of Exposure (MOE) approach it was calculated that a lifelong exposure of only 7 ng PAs/kg b.w. per day would correspond to a MOE of 1:10.000, the threshold of what EFSA considers a low risk. Important food items that need to be considered regarding human exposure are honey and derived products (e.g. pollen), herbal products (risk of contamination with PA-containing plants) and products of animal origin such as milk, dairy products, eggs, meat and animal organs (carry-over from contaminated feed). Depending on the contribution of the specific food category to the daily diet, different limits of detection are required. A high volume product such as milk will require a method enabling very low detection. Moreover, due to metabolic conversion in the animal, novel metabolites can be produced that may be relevant for human exposure as well. The fact that EFSA in their 2011 opinion has identified several dozens of important marker PAs (including many for which no reference standards are available) further adds to the complexity of the task. An overview will be presented of recent advances made in the analysis of PAs in food products, with special attention to the use of high end mass spectrometric approaches for the detection and identification of PAs at trace levels.

Keywords: *Pyrrolizidine alkaloids, metabolites, food products, LC–MS, analytical methods*

J-59
ENHANCED LC–MS/MS–ANALYSIS OF CYLINDROSPERMOPSIN IN PLANT AND FRESHWATER MATRICES USING THE STABLE ISOTOPE DILUTION ASSAY

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Cylindrospermopsin (CYN) is a cyanobacterial secondary metabolite produced by several cyanobacterial species. CYN is a known hepatotoxin and studies have shown its genotoxic potential. Increasing incidences of CYN occurrence in freshwater have been reported all over the world. Thus, several authorities established guidance values for CYN in drinking water in the low µg/L range. Additionally, the CYN uptake in vegetable plants and an accumulation in seafood have been demonstrated in different studies. Therefore, improved rapid methods like antibody based assays to detect this toxin are required to protect consumers´ health by surveillance of CYN in water and food. However, for all newly developed methods a reliable reference analysis for confirmation of the obtained results is mandatory. The application of LC–MS/MS as reference method is very common due to its high selectivity and sensitivity. By application of the stable isotope dilution assay (SIDA), matrix effects in MS detection as well as analyte losses during sample preparation can be compensated leading to analytical results of high accuracy.The aim of the present study was to develop a reliable LC–MS/MS quantitation method for CYN based on SIDA. To date no isotope standard is available for CYN. Thus, in the first instance the biosynthesis of ^{15}N isotope labeled CYN was established using the known CYN producing strain *Aphanizomenon flos-aquae* was carried out. By cultivation of the cyanobacteria on defined media containing $Na^{15}NO_3$ as the only nitrogen source, fully ^{15}N-labeled CYN could be obtained. The biosynthesized U-[$^{15}N_5$]-CYN was isolated from the culture medium using an SPE utilizing graphitized carbon black (GCB) columns followed by preparative LC. Based on the U-[$^{15}N_5$]-CYN, sample cleanup procedures for plant and freshwater samples were developed, followed by a SIDA–LC–MS/MS analysis. For plant material, liquid extraction of freeze dried samples with subsequent cleanup with GCB columns was carried out while water samples were directly applied to the GCB column for analyte enrichment. The applicability of the developed SIDA–LC–MS/MS method was evaluated using naturally contaminated vegetable mustard and spiked freshwater samples. The whole extraction and quantitation method for CYN in vegetable mustard leaves and freshwater yielded low relative standard deviations (< 5%). The LOQ for the examined plant material was approx. 4 µg kg^{-1} fresh weight and, dependent on the biological origin, the LOQ for freshwater was approx. 0.2 µg L^{-1}. Overall the obtained data provide proof for a good performance of the developed method with good sensitivity, low variance and high recovery rates. Thus, the SIDA–LC–MS/MS method is a versatile tool either as reference analysis while evaluating other (rapid) methods or for the direct measurement of CYN in water and foodstuff.

Keywords: *Cylindrospermopsin, LC–MS/MS, Stable Isotope Labeling, Vegetable and Water Analysis*

J-60
UPLC–MS/MS APPROACH FOR THE DETERMINATION OF REGULATED MYCOTOXINS IN CONTROL LABORATORIES

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Mycotoxins are toxic natural food contaminants; their occurrence in food is strictly monitored and regulated worldwide due to possible health risks to consumers and animals. Regulatory limits for aflatoxins, fumonisins, fusariotoxins, ochratoxin A and patulin are already established in some countries, and a number of countries are creating or improving legislation for mycotoxins. As action concentrations and limits are generally quite low, analytical methods for mycotoxins determination have to be both sensitive and specific. For regulatory purposes, it is highly desirable to have a reliable, precise and rapid analytical procedure which is applicable to all regulated mycotoxins in various food commodities. The trend in multi-mycotoxins analysis has been to develop methods covering as many analytes as possible, with a focus on easy and fast sample preparations. Although regulated mycotoxins are often included in such methods, there remain few methods that can easily be applied in regulatory laboratories to perform routine analysis in a wide range of matrices. Many laboratories still apply individual methods for the separate determination of each regulated mycotoxin. These methods generally rely on non-specific detection techniques (e.g. FLD, UV, DAD detectors or GC–MS instrumentation) set by the Association of Official Analytical Chemists (AOAC) or by the European Committee for Standardization (CEN). These methodologies are often time consuming, and are generally less sensitive and less specific than liquid chromatography–mass spectrometry (LC–MS) methods. The present study was conducted in order to fill this gap by developing a sensitive, rapid, and accurate multi-target method for the determination of the regulated mycotoxins; combining a single immunoaffinity column clean-up with UPLC-MS/MS separation and detection. This novel method, designed for the analysis of mycotoxins in regulatory, industrial and private laboratories, was validated for nuts, cereals, bakery products, dried fruits, as well as other foodstuffs. Method optimization was primarily focused on simplifying the extraction and clean-up procedures recommended by the immunoaffinity column manufacturers. This newly developed procedure significantly decreased both the sample preparation time and the solvent volumes used during processing. Matrix-effects caused by the various food matrices and the immunoaffinity columns themselves were fully evaluated in this study. The results obtained indicate the potential use of a single calibration curve for quantitation, based on solvent standards processed by IAC clean-up. This quantitation approach would avoid the need to prepare time-consuming matrix-matched calibration curves for every single analyzed matrix.

Keywords: *Mycotoxins, immunoaffinity clean-up, UPLC–MS/MS*

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J-61
APPLICATIONS OF GREEN AND EFFICIENT EXTRACTION METHODOLOGIES FOR THE DETERMINATION OF MYCOTOXINS IN DIFFERENT MATRICES

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Mycotoxins are secondary metabolites produced by different fungi which contaminate food and feed, causing diseases and disorders in humans and animals. Due to their toxic effects, their presence in food is considered a high risk to health and maximum contents of these compounds have been established in different matrices. Immunoaffinity columns have been commonly used for their extraction. However, simpler, efficient, less contaminant and multiclass extraction methods are demanding. Dispersive liquid–liquid microextraction (DLLME) is one emerging technique for treatment of liquid samples, based on the use of a ternary component solvent system; thus, a mixture of a few µL of an organic extraction solvent, and a small volume of a disperser solvent (miscible with the extraction solvent and water), is rapidly injected into an aqueous sample, resulting in the formation of a stable emulsion. The organic analytes are rapidly extracted into the extraction solvent as a result of the large contact surface between the organic and the aqueous phases. After centrifugation, the organic phase with the analytes of interest is analysed. Another popular treatment is the so called QuEChERS, which presents some advantages, such as simplicity, minimum steps, and effectiveness for cleaning-up complex samples. It involves two steps: (i) extraction based on partitioning via salting-out, involving the equilibrium between an aqueous and an organic layer; and (ii) dispersive solid phase extraction (dSPE) that involves further clean-up using combinations of $MgSO_4$ and different sorbents to remove interfering substances. We have explored the applicability of both methods for the extraction of mycotoxins in different matrices (wines, juices, botanicals, cereals, pseudocereals, cereal syrups, nuts and edible seeds) using different techniques, such as capillary electrophoresis with UV-detection for patulin [1] and capillary HPLC coupled to laser induced fluorescence detection for ochratoxin A [2] or ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) for multiclass analysis [3,4,5]. Combining these environmentally friendly sample treatments and the features of the selected techniques, limits of quantification below the maximum concentrations allowed by legislation were achieved, with satisfactory accuracy, showing the suitability of the proposed methodologies.

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Keywords: *Mycotoxins, DLLME, QuEChERS, foods, beverages*

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J-62
OCHRATOXIN A RESIDUES IN TISSUES OF INDUSTRIAL BROILER AND NON-DESCRIPT (DESI) CHICKEN IN FAISALABAD PAKISTAN

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During a six months study period (May 1 to 14 November), samples of liver, breast muscle and feed were conveniently collected from 131 industrial broiler farms and 52 non-descript (Desi) bird farms around Faisalabad city (Pakistan). Samples from the birds showing no gross changes on visceral organs were rated as healthy while birds having large, pale and or hemorrhagic liver/kidneys were regarded as diseased ones. Out of 131 samples from broiler farms 103 samples were healthy birds and 28 samples were from diseased birds. From 52 samples of Desi bird farms, 39 samples were from healthy and 13 from diseased birds. OTA was extracted by immunoaffinity column technique and quantified by a HPLC-FD method. . In liver of healthy and diseased broilers, the OTA residues could not be detected in 26.21 and 7.14% samples. The highest OTA level in liver of healthy broilers was 1.83 ng/g and it was above 1.0 ng/g in 3.8% samples. In liver of diseased birds the highest OTA levels was 7.68 ng/g and it was above 1.0 ng/g in 24.99 % liver samples In the muscles of healthy and diseased broiler birds the OTA residues could not be detected in 74.76 and 50.00% samples. The highest residue concentration in muscles of healthy broiler birds was 0.35 ng/g in 9.70% birds. In sick birds the highest concentration of OTA in muscles was 0.24 ng/g. In livers of healthy and diseased Desi birds, the OTA residues could not be detected in 46.15 and 7.69% samples. In Desi birds the maximum OTA concentration in the liver of healthy birds was 1.32 ng/g. The residual OTA concentration in liver of healthy Desi birds was above 1.00 ng/g in 2.56% birds. The maximum concentration of OTA in liver of diseased Desi birds was 2.16 ng/g and the levels above 1.0 ng/g were present in 23.07% samples. In muscles of healthy and diseased Desi birds, the OTA residues could not be detected in 87.17 and 46.15% samples. The maximum OTA residual level in muscles of healthy desi birds was 0.09 and 0.21 ng/g in healthy and diseased birds. OTA concentration in feeds was ≤ 20-50, 51-100 and 101-200 ng/g on 87.55, 4.68 and 7.74% broiler farms, respectively. OTA concentration in feeds was ≤ 20-50, 51-100 and 101-200 ng/g on 69.23, 23.07 and 7.69% Desi birds farms. No relation could be developed between OTA concentration of feeds and tissue residue levels in both broiler and desi birds. It could be concluded that OTA was detected more frequently in tissues of non descript (Desi) birds than broiler birds. OTA did not deposited in injurious concentrations in the muscles of healthy or diseased broiler or Desi chicken. However, OTA deposited in the liver of diseased chicken of both types and thus warrant condemnation of grossly abnormal liver.

Keywords: *Ochratoxin, Broilers, Non-descript birds, Residues, Mycotoxins*

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J-63
LINAMARIN IN CASSAVA LEAVES AND ROOTS: ANALYSIS BY LC–MS

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Cassava (*Manihot esculenta Crantz*) leaves and roots are consumed in various parts of the world as a source of protein and starch. However, both cassava leaves and roots contain high amount of toxin called “linamarin”, a cyanogenic glucoside, which causes various diseases or even may cause death to the consumer in severe cases. Cassava leaves and roots are readily available in the exotic shops and are typically consumed as vegetables by African and Asian migrants living in Germany. A simple and efficient linamarin extraction method was developed and a modified protocol for LC–MS was established for identification and quantification of linamarin by using standard linamarin. As a preliminary study, cassava leaves and roots from the exotic food shop (Stuttgart, Germany) and fresh leaves obtained from the cassava plants grown in the university greenhouse were analyzed for linamarin by using LC–MS method. No linamarin was detected in the cassava leaves (from Vietnam and Cameroon) while a reasonable amount of linamarin (13.12 µg/g on fresh weight basis, FWB) was found in peel of the cassava root obtained from the exotic food shop (Stuttgart, Germany). A high amount of linamarin (127.8 µg/g FWB) was detected in the fresh cassava leaves obtained from the greenhouse. To the best of our knowledge, no previous report is available on the linamarin analysis in cassava leaves and roots available in Germany. There is a dire need to analyze all cassava based products for antinutrients and toxins in order to make sure that these are within the safe a limit and these products have been properly processed before getting into the market.

Keywords: *Cassava leaves, roots, linamarin, LC–MS*

J-64
FAST, SIMPLE, AND REPRODUCIBLE SPE CLEANUP OF CORN, WHEAT, AND PEANUT EXTRACTS PRIOR TO HPLC ANALYSIS FOR MYCOTOXINS

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Mycotoxins are toxic secondary metabolites produced by fungi, which can exist in food as a result of fungal infection of crops. Their strong resistance to decomposition and digestion cause mycotoxins to remain in the food chain in meat and dairy products. The analysis of mycotoxins in food and animal feed has been a challenge mainly due to the complexity of food matrices and desired low detection limits. Immunoaffinity SPE cartridges provide a high selectivity for mycotoxins, but they are cost-intensive and the procedure involves multiple steps during cleanup. In this study, we investigate new SPE materials that are designed for sample preparation of mycotoxins in complex food matrices, such as grains and grain products. The proposed methods require homogenization of the matrix, blending of the sample with extraction solvent, and then passing an aliquot of supernatant through SPE cartridge. Performance of the SPE products was evaluated for aflatoxins as compared to immunoaffinity clean-up. Recoveries ranging from 101–108% were obtained for the new SPE material and 78–101% for the immunoaffinity column. The analysis for Deoxynivalenol (DON) was also conducted using the new SPE material with corn, wheat, and peanut matrices. Recoveries for DON were 79–90% for the various matrices. Ochratoxin was also extracted with SPE and average recovery was 108%. These new SPE materials have demonstrated a faster and easier alternative sample preparation method for the quantitative analysis of mycotoxins in various grains and grain products.

Keywords: *Mycotoxins, SPE, LC–MS, Fast Analysis*

J-65
LC–MS/MS MULTI-MYCOTOXIN DETERMINATION AFTER IMMUNOAFFINITY COLUMN CLEAN-UP: A SINGLE-LABORATORY VALIDATION DESIGN TO EVALUATE CONTRIBUTION OF SELECTED FACTORS TO METHOD PERFORMANCE CHARACTERISTICS

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The development and application of LC–MS(/MS) techniques for the determination of multiple mycotoxins continues to be one of the trends in mycotoxin analysis, driven by technical developments in mass spectrometry, simplifying sample preparation. Problems dealing with sample preparation, matrix effects and choice of proper calibration for accurate quantitative analysis still deserve further investigation. Maximum permitted levels for the major mycotoxins, namely aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A (OTA), fumonisins (FB1, FB2), deoxynivalenol (DON), and zearalenone (ZEA) are currently included in the European legislation (1881/2006/EC, 1126/2007/EC), and indicative maximum levels for the sum of T-2 and HT-2 toxins have been recently issued (Recommendation 2013/165/EC). Although not regulated, attention is paid to the occurrence of nivalenol (NIV), another Fusarium toxin that frequently contaminates cereals also in combination with DON. A LC–ESI–MS/MS method intended for quantitative determination of these major mycotoxins in cereals and derived products at levels comparable with EU maximum permitted levels has been developed.The effective co-extraction of the mycotoxins under investigation was achieved in 4 minutes by a double extraction approach, using water followed by methanol. Cleanup of the extract was performed by a new multi-toxin immunoaffinity column (Myco6in1+™, Vicam). An experimental protocol for evaluating analytical performance characteristics through single laboratory validation was designed with the aim to estimate the extent to which selected experimental factors influence the measurement results for each analyte. Raw wheat and maize, corn flakes and maize snacks were chosen as representative matrices for method validation. The validation assay was carried out at 50, 100 and 150% of EU maximum permitted levels for each concerned mycotoxin. Statistical analysis of the results (ANOVA) provided the precision profile of the method (intermediate precision) and the error contributions from various factors: between day variation, influence of matrix composition, and calibration approach (standard or matrix assisted calibration). The same set of experiments permitted experimental determination of limits of detection, limits of quantification and linearity range. Finally, a possible extension of the method to some relevant masked forms of mycotoxins was evaluated by studying the cross reactivity of antibodies of the immunoaffinity column towards mono-glucosil derivatives of DON, T-2 and HT-2 and hydrolyzed forms of FB1 and FB2.

Keywords: *Mycotoxins, cereal foods, immunoaffinity columns, LC–MS/MS, validation*
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J-66
DETERMINATION OF MOULDS AND OCHRATOXIN A IN RAPESEED SAMPLES FROM VOJVODINA – SERBIA

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In this work are presented results of investigations related to mycopopulations of rapeseed samples (20) (from Institute of Field and Vegetable Crops Novi Sad, experimental fields, Rimski Šančevi location – Vojvodina, Serbia, crop 2011), with a special attention to the presence of potentially xerophilic and toxigenic species as well as ochratoxin A (OTA). Mycological examination was performed on untrerated samples and samples treated with Na-hypochlorite solution (4%). Isolation of moulds was done by using two media: Dichloran Glycerol Agar (DG18) and malt agar (MA). It was found that from 20 untreated samples 17 of them were infested with moulds after cultivation on DG18 medium and 11 samples after the growth on MA medium. After the treatment with Na-hypochlorite solution (4%), only 4 samples growing on DG18 and 3 of them on MA were contaminated with moulds. The total number of moulds isolated by using DG18 medium varied from 10.0 to 4.7 x 102 in untreated samples and from 10.0 to 60.0 per g in treated samples. Mould contamination of rapeseed samples was found to be at approximatelly the same level (10.0 to 2.2 x 103/g – in untreated samples, 20.0 to 40.0/g – in treated samples). In isolated mycopopulations xerophilic moulds dominated, especially from genera Aspergillus, Eurotium, and Penicillium. Most of them are well known as producers of various toxic metabolites. Mycotoxicological analyses showed that 16 rapeseed samples contained OTA although at low concentrations (>2.00 to 9.41 µg/kg).

Keywords: *Moulds, ochratoxin A, rapeseed*

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J-67
STUDY OF THE INTERSPECIFIC INHIBITION-COMPETITION PHENOMENA IN BETWEEN OCHRATOXINOGENIC STRAINS PRESENT ON COFFEE BEANS

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The use of bio-competition between little or non-toxigenic strains against toxigenic strains is a strategy to prevent the presence of OTA in coffee. The examples reported in the literature focus on competition between yeasts and bacteria and ochratoxinogenic fungi. Usually, examples of studies on fungi included strains of *Aspergillus niger* aggregate or non-producing Penicillium whose presence inhibited the accumulation of OTA during grape drying (Valero et al., 2007). Biological control by *Trichoderma spp* proved to be an effective method to prevent bunch rot which promotes contamination by other fungi that can be toxigenic (Elad, 1994; Harman et al., 1996; Harman et al., 2004). Reducing OTA production can be obtained by inhibiting the growth and/or production of the toxin or by decontamination due to toxin consumption after its production by another strain. Some examples indeed stated of this phenomenon using Aspergillus section Nigri (Varga et al., 2000; Abrunhosa et al., 2002). It is within this framework that was undertaken trials with co-cultures of strains extracted from coffee to find and explore potential inhibitions and/or competitions against ochratoxinogenic strains. The main objective of this study was to identify possible inhibition and/or competition between Aspergillus strains with very different levels of OTA production: *Aspergillus niger, A. ochraceus, A. westerdijkiae and A. carbonarius. A. westerdijkiae* and *A.ochraceus* were confused during a long time due to their similar characteristics. A. westerdijkiae produced in artificial medium up to 30,000 µg/Kg of OTA. In contact with A. ochraceus which is a weak OTA producer, its OTA production reduced to non detectable. We have shown that there was a real competition between strains of the section Circumdati and the section Nigri. The most interesting couples targets/competitors in terms of reducing the production were *A. westerdijkiae/ A. niger* and *A. westerdijkiae/ A. ochraceus*. We could conclude that in a natural environment, competition in between strains exits and helps to reduce OTA content.

Keywords: *Ochratoxin, coffee, fungi interaction, fungi inhibition*

J-68
ONLINE COUPLING OF COVALENT SOLID PHASE EXTRACTION TO LIQUID CHROMATOGRAPHY FOR AUTOMATED ANALYSIS OF ZEARALENONE

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Zearalenone (ZEN) is a non-steroidal hyperestrogenic mycotoxin that contaminates different crops worldwide. Investigations in several countries of the European Union on the occurrence of ZEN showed 32 % of cereal based foods to be contaminated [1]. Furthermore, notably high levels of ZEN were found in edible oils, especially maize germ oil [2]. Frequent analyses are therefore necessary to control the maximum levels of ZEN in different kinds of food. Thus, the aim of this work was to develop a reliable and cost-efficient SPE-HPLC online coupling for the fully automated analysis of ZEN, particularly for liquid samples like edible oil.
The key principle of the automated technique resides in a SPE step that is not based on a conventional reversed phase SPE but on a recently published covalent SPE approach [3]. During this step, carbonyl compounds as ZEN selectively react with the hydrazine functionalized polymer particles. Interfering matrix components are subsequently washed off. In a final step ZEN is released from the particles and quantified by HPLC with fluorescence detection.
This talk will summarize the chemical and technical development including the evaluation of different polymer particles, information on recovery and re-usability rates and the technical setup. This is the first work to explore whether a SPE based on covalent interaction can be coupled to HPLC and the first approach for fully automated analysis of zearalenone in edible oil.

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[3] Siegel, D., Andrae, K., Proske, M., Kochan, C., Koch, M., Weber, M. and Nehls, I., 2010. Dynamic covalent hydrazine chemistry as a selective extraction and cleanup technique for the quantification of the Fusarium mycotoxin zearalenone in edible oils. J Chromatogr A 1217: 2206–2215.

Keywords: *Zearalenone, SPE-HPLC online coupling, covalent SPE, hydrazine, edible oil*

J-69
NOVEL METHOD FOR THE ACCURATE MEASUREMENT OF ZEARALENONE AND DEOXYNIVALENOL IN CEREALS

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Mycotoxins have been defined as “fungal metabolites which when ingested, inhaled or absorbed through the skin cause lowered performance, sickness or death in man or animals, including birds”. The main mycotoxin-producing fungal genera are *Aspergillus* (e.g. aflatoxins and ochratoxin A), *Fusarium* (e.g. trichothecenes, zearalenone and fumonisins B1 and B2) and Penicillium (e.g. ochratoxin A and patulin). Zearalenone and deoxynivalenol are co-produced by *Fusarium graminearum* and are widely distributed in a variety of cereals, including wheat, maize, barley and rice. Both toxins can have a serious impact on human and animal health and productivity. Zearalenone is a potent oestrogenic compound and deoxynivalenol (vomitoxin) is toxic towards the alimentary tract. It is very important that all companies within the food and feed sectors are able to test for mycotoxins at all points along the global supply chain, to ensure that food and feeds are safe to consume and meet with international regulations. Currently available testing methods can be divided into two groups. Techniques such as Lateral Flow, ELISA, Fluorometric, TLC and HPTLC are rapid and affordable screening procedures but lack the accuracy of expensive and complex technologies such as HPLC, UHPLC and LC–MS/MS. The ToxiMet System is rapid, affordable, user-friendly and accurate, combining the best properties of current technologies. It comprises ToxiSep® clean-up cartridges, ToxiTrace® cartridges (on which the toxins to be analysed are immobilised) and the ToxiQuant® instrument which accurately measures the immobilised toxins. The System is specifically designed to be used by non-scientists at any point within the food supply chain. In this presentation the application of the ToxiMet System to the analysis of zearalenone and deoxynivalenol (a trichothecene) is reported. Methods for the extraction and clean-up of cereal samples will be described together with the immobilisation of zearalenone and deoxynivalenol on a ToxiTrace® cartridge. Procedures developed for the fluorescent enhancement of both toxins, by chemical manipulation and derivatisation, will also be reported. It will be demonstrated that the employment of a combination of fluorescence spectrometry and chemometrics, within the ToxiQuant® instrument, enables the simultaneous measurement of both toxins and the production of results of comparable accuracy to HPLC.

Keywords: *Analysis, zearalenone, deoxynivalenol, cereals, ToxiMet*

J-70
DETERMINATION OF DEOXYNIVALENOL BY RAPID SCREENING TESTS AND COMPARISON OF RESULTS WITH LC–MS DATA

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Deoxynivalenol (DON) is the most frequently occurring Fusarium mycotoxin that can contaminate cereals grown under the mild climate conditions. DON is commonly considered to be a marker of FHB (Fusarium head blight) disease of cereals. Nowadays, simple, rapid and cost-effective screening tests are commercially available and widely used for determination and even quantification of DON. For the purpose of presented study four types of lateral flow assays (LFA) were tested for their suitability for determination of DON in cereals. Due to general tendency to overestimate results obtained by LFA, which are commonly caused by structurally similar compounds and matrix co-extract, recoveries and cross-reactivities for the main DON metabolites were tested for following assays:
i) RIDA® QUICK DON (R-Biopharm; Germany);
ii) QuickScanTM (EnviroLogix; USA);
iii) Charm ROSA® (Charm Sciences Inc.; USA);
iv) Reveal® Q+ for DON (Neogen Corporation; USA).
In total, 28 samples of barley, wheat and rye cereals and three certified reference materials (wheat, maize, barley) were analyzed by screening tests mentioned above. Results obtained by these tests were compared to those obtained by the UHPLC–MS/MS accredited analytical method (ISO 17025). Recovery of DON was tested using artificially contaminated sample of wheat blank. DON recoveries 71%, 115%, 60%, 91% were observed for RIDA® QUICK DON, QuickScanTM, Charm ROSA® and Reveal Q+, respectively. Cross-reactivity to DON-3-glucoside and 3-acetylDON were observed in all cases of tested LFA and were in range 14–197%. Results obtained by screening tests and UHPLC–MS/MS methods were in a good agreement, and thus data produced by these assays could be considered as reliable for screening purposes. Standard deviation calculated from parallel samples was below 10% for all tested samples. An average percentage balance of results obtained by rapid tests and UHPLC–MS/MS were calculated a s 132%, 125%, 88%, 104% for RIDA® QUICK DON, QuickScanTM, Charm ROSA® and Reveal Q+, respectively.

Keywords: *Mycotoxins, deoxynivalenol, rapid screening tests, crossreactivity, UHPLC–MS/MS*

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J-71
DEVELOPMENT AND VALIDATION OF METHODS FOR THE AUTOMATED QUANTITATIVE ANALYSIS OF MYCOTOXINS IN VARIOUS COMMODITIES BY IMMUNOPREP® ONLINE CARTRIDGES

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IMMUNOPREP® ONLINE immunoaffinity cartridges have been developed by R-Biopharm for the automated clean-up and analysis of mycotoxins by HPLC or LC–MS/MS in various commodities. The online immunoaffinity cartridges are used in conjunction with the Symbiosis™ Pico HPLC system from Spark Holland and combine automated online sample clean-up with quantitative analysis of mycotoxins. The affinity cartridge contains a monoclonal antibody that is specific for the target analyte, coupled to a hydrophilic polymer that can withstand high pressure enabling it to be used in series with an analytical column. Following extraction of the toxins from the sample with solvent, the extract is filtered, diluted and transferred to an autosampler vial. The diluted extract is automatically injected onto the immunoaffinity cartridge and any toxins present in the sample are retained by the antibody in the cartridge. Unbound matrix material is removed by washing the cartridge and sending the resulting wash to waste. Subsequently, the toxins are released from the antibody following online elution. During elution, the immunoaffinity cartridge is switched online with the analytical column and the complete elution fraction from the cartridge is quantitatively analysed for the particular mycotoxin by HPLC. The technology is highly innovative and enables the cartridge to be incorporated directly online with an HPLC system. A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the immunoaffinity cartridge, reducing the time taken for subsequent sample clean-up to almost zero. The use of an online immunoaffinity cartridge reduces labour, consumables and solvents whilst improving traceability, accuracy and reducing human error. The immunoaffinity cartridge offers highly specific, sensitive, rapid and automated analysis for mycotoxins in a wide range of food matrices, such as peanuts, cereals, dried fruit and spices. Under optimised conditions, the immunoaffinity cartridge is reusable for 20 analysis including standards for calibration, method and/or instrument blanks, QC and test samples. This level of reuse has been found to offer improved quality and reduction of re-analysis, because each cartridge is qualit y controlled prior to the analysis of the samples. In this presentation, the technology behind the IMMUNOPREP® ONLINE immunoaffinity cartridges will be discussed along with how the cartridges are used in conjunction with the Symbiosis™ Pico system. Finally, methods and validation results for the automated analysis of mycotoxin in various commodities will be presented.

Keywords: *Immunoaffinity, HPLC, LC–MS/MS, mycotoxins, immunoaffinity*

NANOPARTICLES

(K-1 – K-12)

K-1
PREPARATION AND CHARACTERIZATION OF CHIOS MASTIC GUM FRACTIONS BEFORE AND AFTER ENCAPSULATION IN LIPOSOMES BY THREE DIFFERENT METHODS

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Abstract Chios mastic gum, the resin obtained as an exudate from the trunk and branches of Pistacia lentiscus L var. chia, has found extensive use in pharmaceutical products and as a nutritional supplement. The oral absorption of crude resin (contained a high percentage of an insoluble and sticky polymer of poly-β-myrcene) is poor due to its low water-solubility and reduces the bioavailability of the contained active compounds [1]. A total mastic extract without polymer (TMEWP) was prepared after removal of the contained insoluble polymer in order to ameliorate solubility and enhance in vivo activity. To further characterize potential active mastic constituents, the TMEWP was separated into an acidic and a neutral fraction. To overcome the drawbacks of ME, the selection of a suitable carrier is very necessary and crucial. Three different methods of preparation, thin film evaporation (TFE) [2], solid lipid nanoparticles (SLN) [3], and ethanol injection (EI) [2] used for the preparation of liposomes consisting of phosphatidylcholine (PC) and cholesterol (CH). The effect of PC:CH molar ratio on the percentage of mastic extract encapsulated was investigated. Mastic gum extracts components-liposomes interaction was studied using Fourier transform infrared (FT–IR) spectroscopy and differential scanning calorimetry (DSC) [4]. The effects of different preparation methods on the physicochemical properties of lip osomes were evaluated by means of surface morphology by field emission scanning electron microscopy, zeta potential and size distribution using a Zetasizer and particle size analyser, respectively. Antioxidant and antimicrobial activity of the fractions (acidic and neutral) before and after encapsulation in liposomes also studied.

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Keywords: *PC:CH liposomes, mastic gum extracts, encapsulation, liposome's characterization*

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K-2
SINGLE PARTICLE ANALYSIS OF NANOMATERIALS WITH AGILENT ICP–MS

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In the present work the analysis of nanomaterials composed from different elements has been carried out under the single particle mode and with the use of Agilent ICP–MS. The method allows the determination of the nanoparticles mass concentration in the sample but also the evaluation of their size distribution and median size.

Keywords: *Nanoparticules, ICP–MS, Single particle analysis*

K-3
PRELIMINARY ASSESSMENT OF E171 PARTICLE SIZE DISTRIBUTION: THE IMPORTANCE OF PH AND DISPERSION METHOD

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In 2011, the European Commission has published the following recommended definition for the term nanomaterial: "Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm. The practical application of this definition in legislation is being hindered by the lack of analytical methods which can determine whether a material should or should not be classified as a nanomaterial under the terms of this definition. Currently there is no single analytical method or instrument able to fully satisfy the requirement of the definition but a variety of methods exist which, in combination, offer the possibility of addressing the problem. The food industry will particularly affected by this, in fact regulation 1169/2011 ("On the provision of food information to consumers") provides labelling obligation for nanoingredients present in food. A relevant example of this is TiO_2 which, in the form of EU approved white colorant E171, is widely used in processed food and in particular in confectionary products. Currently the specification for alimentary grade TiO_2 does not foresee a detailed characterisation of particle size distribution although there is evidence in the literature that part of the titanium dioxide in E171 may be in nano form [1]. Therefore there is a need for methods for the simultaneous detection and quantification of nano-titania in food matrices. Compared to other consumer products, the analysis of nanomaterials in food poses a number of difficulties due to the tremendous complexity of the matrix and the fact that sample preparation can greatly alter the resulting particle size distributions. In this work results will be presented from a preliminary study of E171 and E171-containing products to assess the effect of pH, sonication and incubation in acid and basic environment on the particle size distribution. Preliminary size characterization based on Centrifugal Liquid Sedimentation provide clear evidence that all the above factors can modify the apparent particle size distribution and this must be taken into account in developing and validating analytical methods. These studies have also highlighted the urgent need for new reference materials to be used in the development and application of analytical methods for detecting and quantifying nanomaterials in food.

[1] Weir, A., et al., Titanium dioxide nanoparticles in food and personal care products. Environmental Science and Technology, 2012. 46(4): p. 2242–2250.

Keywords: *Titanium dioxide, E171, nanoparticles, regulations*

K-4
NANOTECHNOLOGY IN PRODUCTION OF KETCHUPS AND SEASONINGS

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It is known that one of the main components, defining organoleptic characteristics of ketchups and seasonings, which is their color, a smell and taste, spices are. Into number of these products enter both rather cheap and easily available, and very expensive and growing only in some remote places of the globe. Therefore the cost of this popular food becomes very essential. Besides it is known that many spices in considerable (5–20 grams of spices on 100 grams of a ready-made product) quantities negatively influence functioning ventricular - intestinal path, a cardiac muscle and other systems of a human body. However, use of these spices in smaller doses negatively affects on organoleptic ready-made products and reduces their popularity at consumers. Research of the production technology of ketchups and sauces with application of nanoprocessing of spices used in a compounding was conducted. As a result of the obtained experimental data it is defined that qual ity of ready-made products didn't change, the amount of using spices significantly decreased and, as a result, finished goods cost also decreased. So, at production of the tomato ketchup, one of the most popular seasonings to a large number of various meat and fish dishes, the consumption of the most expensive spices decreases more than by 2.5 times, and the cost of its production decreases by 40%.

Keywords: *Nanotechnology, spices, organoleptic, ketchups, seasonings*

K-5
CHARACTERIZATION OF SILVER NANOPARTICLE INTERACTIONS WITH SERUM AND WHEY PROTEINS

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Introduction: The application of nanomaterials (e.g. silver nanoparticles) has recently become an issue in the food sector. Based on their antibacterial properties silver nanoparticles are applied as dietary supplement or in food contact materials. Migration of silver in nanoform into food matrices or contamination of food with nanosilver might result in a significant exposure of the consumer via the oral route. Therefore development of suitable tools to identify nanoparticles in food matrices is of utmost importance. A further important issue is the possible alteration of the primary characteristics of nanoparticles upon interaction with food constituents. Nanosilver is known to form a so called "protein corona" with proteins in the surrounding medium. Therefore the analysis of the interaction of silver nanoparticles with food components (e.g. proteins) is of high importance in order to increase our knowledge on the behavior of nanoparticles in the gast rointestinal tract.
Methods: The interaction of silver nanoparticles with proteins was investigated with size exclusion chromatography (SEC) and dynamic light scattering (DLS) after incubation of isolated proteins with silver nanoparticles. SDS–PAGE was applied to qualitatively analyze the binding of serum and whey proteins to nanoparticles. Additionally changes in characteristic surface plasmon resonance of silver due to interaction with proteins were measured by means of UV-vis spectroscopy.
Results: It could be shown that silver nanoparticles interact with isolated whey and blood serum proteins (BSA, fibrinogen and beta-lactoglobulin) as well as fetal calf serum and unprocessed whey. Commercially available silver nanoparticles of different sizes (20 or 60 nm) and coatings bound BSA, beta-lactoglobulin and fibrinogen with different affinities. SDS–PAGE analysis indicated that fibrinogen is bound more strongly than the other two proteins. This was also confirmed by DLS measurement showing the most significant increase in the hydrodynamic diameter upon interaction with fibrinogen. The formation of a protein corona with BSA and beta-lactoglobulin was furthermore confirmed by SEC. A shift in surface plasmon resonance and an alteration of surface charge (zeta potential) of silver nanoparticles was observed after interaction with the proteins studied. SDS–PAGE protein patterns of the protein corona formed after incubation with fetal calf serum and whey indicated select ive binding of certain proteins.
Conclusions and outlook: Interactions of silver nanoparticles with proteins are protein specific and depend on the chemical properties of the nanoparticles. A combination of various analytical methods is necessary to characterize these processes in detail. To increase our knowledge on the behavior of nanoparticles in a food matrix, the studies on the interaction of silver nanoparticles will be extended to complex food matrices, e.g. milk.

Keywords: *Protein corona, silver nanoparticles, whey proteins, serum proteins*

K-6
CHARACTERIZATION OF NANOPARTICLES USING ICP–MS – ADVANTAGES AND CHALLENGES FOR NANOPARTICLES IN FOOD

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The analysis of Nanoparticles (NPs) has become one of the hot topics in food safety due to the risk associated with NPs and their affect on human health. Although many everyday products, including many foods, contain NPs either intentionally, naturally or through contamination, detailed knowledge about potential risks or hazards is still unavailable. In order to leverage the potential of ICP-MS for the analysis of NPs, two approaches have been developed in recent years:
1. Hyphenation of an appropriate separation technique like Field-Flow-Fractionation (FFF), or
2. Direct analysis of nanoparticles using spICP–MS.
FFF has a separation principle based on the differing mobilities of different particle sizes in a laminar liquid flow. FFF is compatible for particle sizes in the low nm to low µm range and is thus perfectly suited for the separation of different NPs. In comparison, spICP–MS is able to analyze NPs directly based on the signal intensity of single p article events in the plasma which are directly proportional to the size of the NP. This direct approach greatly simplifies the experimental set-up. ICP–MS instrumentation with outstandingly high detection sensitivity extends the particle size limit of detection into the low nm range. In this presentation, the theory and typical requirements of both techniques are going to be presented. The key benefits and drawbacks of each technique are going to be illustrated with samples that contain nanoparticles of different structure and size and the challenges that lie ahead for characterization of NPs in food products will be discussed.

Keywords: *Nanoparticles, ICP–MS, Field-Flow-Fractionation, spICP-MS*

K-7
EXPLORING THE USE OF INULIN AS A VEHICLE FOR THE DELIVERY OF COLONIC DRUGS

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Inulin is a naturally occurring polysaccharide found in many plants. It consists of β 2-1 linked D-fructose molecules having a glucosyl unit at the reducing end. Various inulin and dextran hydrogels have been developed that serve as potential carrier for introduction of drugs into the colon. Colon cancers are difficult to treat because oral administrated drugs are absorbed at the stomach and intestine levels and they do not reach colon; in addition, intravenous administrated drugs are eliminated from the body before reaching colon. Because inulin is not absorbed in the stomach or in the intestine, and inulin is degraded by colonic bacteria, drugs encapsulated in inulin-coated vesicles could be specifically liberated in the colon. Therefore, the use of inulin-coated vesicles could represent an advance for colon cancer treatment. The encapsulation of several antitumoral drugs in inulin nanoparticles and their release and activity was studied in several colon cancer cells in cultures. An optimization study for the synthesis of inulin nanoparticles and drug release by inulinase is presented. Microspheres were prepared from a totally cinnamoylated derivative of inulin (INCN). Microspheres without drug were obtained by dropping a INCN solution with distillate water until turbidity. For MTX loading a concentrate solution of this drug in sodium carbonate (pH 8.6) was added drop by drop to the INCN-Tween 20 solution. The mixture was maintained at room temperature with stirring for 3 h with the formation of a pale white precipitate. After this time the precipitate was filtered and extensive washed with sodium carbonate (pH 8.6) followed with distillate water to removed unincorporated MTX. Microspheres were dried at room temperature on P2O5 at reduced pressure. The amount of drug entrapped in INCN resulted to be 25% (*w/w*) as determined after extensive extraction and UV/Vis analysis.

Keywords: *Inulin vesicles, colon cancer, methotrexate, controlled drug delivery*

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K-8
HIGHLY ORDERED SBA-15 FOR RETENTION OF POLYPHENOLS FROM RED WINE

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Since the discovery of highly ordered M41S family mesoporous molecular sieves in 1992, considerable attention has been focused on tailoring the chemical composition of these materials via the surfactant templated hydrothermal synthesis. Highly ordered large pore SBA-15 was synthesized by co-condensation of tetraethylorthosilicate (TEOS) and 3-aminopropyl-triethoxysilane (APTES) using an amphiphilic block copolymer as the structure-directing agent and characterized by BET, EDX, TGA-DSC and SEM. This mesoporous material exhibits a type IV nitrogen adsorption-desorption isotherm and has a specific surface area of 1108 m2/g. The adsorption properties of the calcined mesoporous silica SBA-15 material, as a consequence of the presence of silanol groups that are active sites for adsorption, were evaluated in the clarification process of two types of red wines. The purpose of this study was the investigation of the total polyphenols content (reduction), the selectivi ty and stabilization for some compounds (phenolic acids, epicatechin, catechin) and the chromatic characteristics of wine. HPLC chromatograms exhibits the retention of quercetin and trans-resveratrol, catechin, epicatechin, rutin, and phenolic acids. The diminution of polyphenolic compounds content in red wines avoid the oxidative browning process without modification of wine color. Due to their beneficial to human health thanks to antioxidative, antiinflamatory, antiviral, antihypersensitive, cardioprotective, anticarcinogenic and antimutagenic properties the polyphenolic extract might prove interesting as pharmaconutrients or as dietary sources.

Keywords: *HPLC, mesoporous, polyphenols, SBA -15, wine*

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K-9
ONE-POT NANOCOMPOSITE SYNTHESIS, CHARACTERIZATION AND APPLICATION

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Synthesis, characterization and application of Au-PANI-Calix and Au-PANI-Nap nanocomposites, is reported herein. An easy template free green synthesis is proposed and discussed for easy expediency. The nanocomposites were immobilized on screen printed electrode, Au-PANI-Calix modified electrode shows sensitive and selective determination of Cu^{2+} with a detection limit of 10 nM, lower than the value allowed by US-EPA, while Au-PANI-Nap modified electrode shows sensing application for H_2O_2 with a detection limit of 1 µM. The copper detection is facilitated for accessible ligation with 4-sulfo-caix[4]arene, so as the Cu(II)-Calix complex formed. The method is favorable to detect Cu^{2+} and H_2O_2 at physiological pH that can further exploit it to biological applications. Electrochemical method has the advantage of using less sample volume and preparation. Henceforth, the nanocomposite materials are good asset to run the application at trace level. Electrode were stable for more than 2 months and no interference from ions K^+, Ni^{2+}, Co^{2+}, Pb^{2+}, Cr^{3+} was observed on the Au-PANI-Calix modified electrode.

Keywords: *Gold nanocomposite, green synthesis, electrochemical sensor*

K-10
ZINC-NANOPARTICLES EFFECTS ON TOXIGENIC FUNGI DEVELOPMENT

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Toxigenic fungi cause foods deterioration and production mycotoxins – a public health problem. To meet the need of reducing and/or inhibiting fungi growth, it is essential to carry out studies on new antifungal agents that can assist in the current control strategies. Nanoparticles have special attention due to their physical/chemical properties, especially on new products with antimicrobial activity. In our study, antifungal and antimycotoxin properties of zinc oxide nanoparticles (ZnO-NPs) were evaluated against toxigenic strains of *Fusarium graminearum*, *Penicillium citrinum* and *Aspergillus flavus*. In addition, their activity on conidia production, hyphae morphological alterations, mortality and reactive oxygen species (ROS) production were verified. The ZnO-NPs were synthesized and characterized by X ray diffraction (XRD) and transmission electron microscopy (TEM) image. Antifungal tests were performed by agar dilution method (at 0, 10, 25, 50 and 100 mM) and the treatment efficacy was evaluated at 8 days. For mycotoxin production, *F. graminearum*, *A. flavus* and *P. citrinum* strains were grown on potato dextrose agar containing 100 mM ZnO-NPs and quantified by thin layer chromatography. Conidia production was evaluated by Neubauer chamber count by light microscope. To investigate if there were changes in the ZnO-NPs treated fungi structures (hyphae morphology / mortality / increase ROS), the scanning electron (SEM) / light / confocal-light microscopy techniques were utilized, respectively. Analysis of variance (ANOVA) followed by Bonferroni post-test were applied for data statistic analysis. The XRD spectrum confirmed the presence of ZnO and TEM image showed NPs mean diameter (30 nm). ZnO-NPs was able to completely inhibit P. citrinum growth of the highest concentration applied, and reduced F. graminearum and *A. flavus* (Control: 59 and 38; Treated: 29 and 28 mm) strains, respectively. The Zn-NPs also inhibited the ability of *F. graminearum* to produce deoxynivalenol. On the other hand, *A. flavus* and *P. citrinum* were able to produce aflatoxin (AFB1) and citrinin, respectively. Despite this, the amount of AFB1 produced after treatment was significantly lower than Control. The conidia production was reduced in 81.2 and 73.9% for treated *F. graminearum* and *P. citrinum*, respectively. SEM analyses allowed to observe alterations in the structures (deformed; broken and unusual bulges hyphae) of the fungi grown in the ZnO-NPs treated medium. ZnO-NPs hyphae were blue stained, suggesting the presence of dying hyphae that undergo plasma membrane lysis, unlike of normal hyphae (Control) that remain with their natural color. Our results also pointed out an increase on ROS production in the ZnO-NPs fungi hyphae treated, observed as a stronger fluorescence intensity. Ours data suggest that Zn-NPs was efficient against toxigenic fungi tested and could be further studied as effective fungicides in agricultural crops and for food safety applications.

Keywords: *Zinc, nanoparticles, fungi, mycotoxins, scanning microscope*

K-11
PRODUCTION, MEASUREMENT AND TESTING OF NANO SIZE ELEMENTAL SELENIUM

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Elemental selenium nanoparticles can be prepared by chemical or biotechnological methods. Some bacteria can reduce the selenite to elemental selenium and can store it inside the cell. The selenium has a spherical shape and very uniform size. Generally the size of spheres is between 100 and 500 nm and the size is depending on the applied microorganism. The application of yogurt bacteria, *Lactobacillus, Bifidobacterium* species and *Streptococcus thermophilus* is especially good and practical for production of amourphus, red elemental selenium particles. A nanosize red elemental selenium sphere has surprisingly good bioavailability and very low toxicity according to our animal and human studies. Therefore the selenium enriched yogurt is an ideal method for selenium supplementation. Nowadays food supplements and different diary products contains selenium enriched yogurt, therefore the speciation of selenium has new challenges, we have to measure the elemental sele nium as well. The measurement of elemental selenium from complicated matrix is challenging. We applied a selective extraction method for the separation of elemental selenium. In this extraction CS_2 is an appropriate solvent, because it selectively dissolves the elemental selenium. After separation of the phases, the CS_2 can be evaporated and the remaining elemental selenium can be dissolved with concentrated nitric acid. The dissolved selenium in the form of selenite can be measured by suitable spectroscopic methods like ICP–OES, ICP–MS, GFAAS or AFS. In the presetation the method of production, purification and measurement of elemental selenium particles, the result of animal experiments and human studies will be presented. According to the results a modell was developed for better understanding of the unique behavior (low toxicity and good bioavailability) of selenium nanoparticles in biological systems.

Keywords: *Elemental selenium, nanoparticles, yogurt bacteria, toxicity, animal and human studies*

K-12
ACCURATE NANOPARTICLE ANALYSIS USING FLOW- AND CENTRIFUGAL FFF

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Nanoparticles (ENPs) are of widespread use for industry and increasing manufacturing, processing and utilization of ENPs lead to the presence of unnatural nanomaterial in consumables [1]. The EU commission already published a number of regulation and risk assessments of nanoparticles and definitions in 2011 [2] and more recently updated the cosmetics directive for nanoparticles [3]. Therefore the characterization of nanoparticles is of upmost interest with the need for straight-forward analysis technology. The use of Field-Flow-Fractionation (FFF) being the latest technology developed for accurate and sensitive separation (fractionation) of nanoparticles, biomolecules and polymers coupled to suitable detectors is steeply increasing. The separation is achieved within flow streams in an unpacked channel. A force perpendicular to the sample stream line, like a second flow, thermal, centrifugal field or gravitation, is applied to facilitate separation. Thus, a whol e FFF-family (platform) is generated according to the separation field applied, which enables the user to choose the most appropriate technique for a given task. The poster presented here focuses on the analysis of nanoparticles by FFF, in particular by asymmetric Flow Field-Flow-Fractionation (AF4) and Centrifugal FFF (CF3) coupled to a dynamic light scattering detector (DLS). By the combination of separation and analysis within one method one obtains detailed information about the size, size distribution and overall intensity of the sample (resp. correlation of size and an external input signal). Furthermore, the real-time analysis allows the user to directly monitor analysis progress and results during measurement. Thus, the coupling of DLS with AF4 facilitates a straightforward on-line size-analysis setup, which is exemplarily shown for latex nanoparticle standards. Any overestimation of hydrodynamic radii commonly accompanied by batch measurements (due to size averaging) can therefore be neglected and a time-resolved, true size-distribution is obtained.[4]

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Keywords: *Field-Flow Fractionation, characterization of nanoparticles, FFF, size distribution, AF4*

NOVEL FOODS AND SUPPLEMENTS

(M-1 – M-10)

M-1
PERMEABILITY OF ISOFLAVONES IN FOOD SUPPLEMENTS CONTAINING SOY, RED CLOVER AND KUDZU ACROSS CACO-2 CELL MONOLAYERS

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Isoflavones are phenolic compounds structurally similar to 17-β-estradiol. This confers them hormonal effects, including the ability of binding to estrogen receptors and modulating hormone-dependent processes. Epidemiological evidences suggest isoflavones have positive effects in prevention of some forms of hormone-dependent cancers, cardiovascular disease, osteoporosis, and adverse menopausal symptoms. Driven by these putative health benefits, a plethora of products containing isoflavones have come on to the market, with great consumer demand. Food supplements preparations generally contain extracts from soy, red clover, and/or kudzu. Several studies have shown a great variability in marketed products regarding the concentration and source of isoflavones, stressing the need of standardization and quality control of these products, considering its therapeutic use. Additionally, the biological effectiveness of these bioactive compounds depends greatly on the intestinal bioavailability, variable between isoflavones. The aim of this study was to investigate the permeability of the different isoflavones present on selected supplement extracts across human intestinal epithelial Caco-2 cell monolayers. Four different products (1 tablet and 3 capsule formulations) with different isoflavones sources (Thai kudzu, red clover, standardized isoflavonoids from soy, and soy extract) were purchased from local retail and health stores. Samples were ground to a fine powder and dispersed in C18 sorbent, cleaned-up with water, and isoflavones eluted with methanol–water (9:1, v/v). Isoflavones concentration in the samples were determined by RP–HPLC/DAD. Concentrations of 9 isoflavone glycosides and aglycones (puerarin, daidzin, genistin, daidzein, genistein, glycitein, formononetin, prunetin, and biochanin A) were determined. The extracts were further used for the permeability assays. Permeability assays were performed in phosphate-buffered saline (PBS) at 37°C from apical to basolateral side. Initially, 500 µL of the sample extract was added to the apical side of the cells. 500 µL were taken from the basolateral side at 15, 30, 60, and 120 min, and from the apical side at the end of the experiment, and isoflavones determined by HPLC. The apparent permeability coefficients (Papp) of the isoflavones were calculated. After 120 minutes, the aglycones were transported through the Caco-2 monolayer, in the apical to basolateral direction, at higher rates than the glycosides.

Keywords: *Food supplements; Isoflavones; permeability; Caco-2*

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M-2
COMPARISON OF PHYSICOCHEMICAL PROPERTIES OF GELATINS EXTRACTED FROM SKINS OF TWO FRESHWATER FISH

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This study was conducted to extract gelatins from two freshwater fish skins: catfish (Clarias gariepinus) and catfish (*Pangasius sutchi*) and determine the physicochemical properties of extracted gelatins and compare with commercial gelatin from bovine skin. Result showed that two extracted gelatins had lower protein content and higher lipid, moisture and ash content compared to commercial gelatin. The number of imino acids (proline and hydroxyproline) of catfish (*Pangasius sutchi*) skin gelatin (18.05) was much higher than catfish (*Clarias gariepinus*) gelatin (17.24). Both catfish gelatin were snowy-white and light-textured material. Microscopic examination of catfish (*Clarias gariepinus*) gelatin gel showed a few more voids than the catfish (*Pangasius sutchi*) gelatin gel. A number of interwoven porous protein fibers were observed which is typical of gelatin and other hydrocolloids, making them resemble a sponge-like matrix. FTIR spectra of two catfish gelatins exhibited majo r adsorption bands in amide band region. The major absorption bands of catfish gelatins were found at 3309–3310 cm^{-1} (amide A), 2926–2928 cm^{-1} (amide B), 1643–1645 cm^{-1} (amide I), 1555–1563 cm^{-1} (amide II) and 1239–1241 cm^{-1} (amide III). The results indicated that high quality gelatin can be produced form (Pangasius sutchi) and (Clarias gariepinus) processing by-products.

Keywords: *Gelatin, fish skin, physicochemical properties, freshwater fish*

M-3
MULTI-TEMPERATION DESORPTION IONIZATION STRATEGY FOR HERBAL SUPPLEMENT CHARACTERIZATION USING DART–MS

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Direct analysis in real time (DART®) mass spectrometry provides a rapid means to profile the chemicals present in samples. Utilizing the sequential measurement of mass spectra at a series of increasing gas temperatures results in generation of mass spectra that provide a more comprehensive view of the composition of the sample. In the case of herbal supplements we complete positive and negative ion detection at four different temperatures in order using NIST reference standards for comparison with commercial products. The use of these thermal profiles for characterization of supplements and its potential use in determination of contamination or adulteration will be discussed using several popular herbal products. Application of the thermal profile method to determination of supplements contaminated with other unrelated herbal products will be made. Expansion of the method to analysis of different chromatographic extracts that might be used to clean-up the sample prior to DART–MS will highlight the utility of simple methods in making higher quality results.

Keywords: *Herbal Supplement, Ambient Ionization, DART, mass spectrometry*

M-4
TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME-OF-FLIGHT MASS SPECTROMETRIC DETECTION FOR TARGET ANALYSIS AND NON-TARGET SCREENING OF ANABOLIC STEROIDS IN FOOD SUPPLEMENTS

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Czech Agriculture and Food Inspection Authority (CAFIA) is responsible for official control of foods which are placed on a Czech and/or European Union (EU) market. In recent years a number of food supplements have been introduced to the Czech market with an aim to attract consumers. However, as noticed by World Anti-Doping Agency (WADA), 17% of food supplements produced worldwide may be contaminated with anabolic steroids. Non-supervised use of these compounds may lead to serious health complications (e.g. kidney or liver cancer) and therefore their application otherwise than for therapeutic and medical use is banned. CAFIA approach to analysis of food supplements consists of *(i)* target analysis of 28 anabolic steroids and *(ii)* non-target screening whereas both are performed using two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC–TOF MS). While target analysis is based on a comparison of collected chromatographic and mass spectrometric data with an analytical standard, non-target screening is performed by processing the data using a ChromaTof software and comparison of deconvoluted spectra of unknown peaks with a NIST library. For sample extraction and clean-up simple and effective method developed and validated in our laboratory is applied. Results of the monitoring programme with examples of positive samples containing anabolic steroids (i.e. dehydroepiandrosterone, 1,4,6-Androstatrien-3,17-dione etc.) together with the basic performance characteristics of the analytical method will be shown in the contribution.

Keywords: *GC×GC–TOFMS, food supplements, anabolic steroids*

M-5
ANALYSES OF FAT-SOLUBLE VITAMINS, CAROTENOIDS AND LIPIDS BY SUPERCRITICAL FLUID CHROMATOGRAPHY WITH SUB-2µM PARTICLE COLUMNS

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UltraPerformance Convergence Chromatography™ (UPC2) is a separation technique that uses compressed carbon dioxide as the primary mobile phase. It takes advantage of the unique physical properties of compressed carbon dioxide (at or near supercritical state), sub-two micron particle chromatography columns and advanced chromatography system design to achieve fast and reproducible separation with high efficiencies and unique selectivity. These improvements lead to new interest in applying this technology to various industrial analytical areas, especially those areas where normal-phase liquid chromatography (NP LC) has been commonly used, such as fat-soluble vitamins (FSV), carotenoids, and lipids. Nine representative FSV and carotenoids have been successfully separated simultaneously by UPC2 within four minutes on a single C18 column. These FSV and carotenoids include vitamin A acetate and palmitate, alpha-tecopherol and its acetate, vitamin D2, vitamin K1 and K2 (MK4), beta-carotene and lycopene. The repeatability (n=6) of all the nine compounds was less than 0.25% in retention times (RT) and less than 2.6% in peak areas. The investigation of lipids separation by UPC2 showed that Bridged Ethylene Hybrid (BEHTM) silica columns provided the best separation of lipid classes among the Flouro-Phenyl, 2-EP, and BEH UPC2columns. The lipid classes investigated include ceramides, sphingomyelin, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, lyso-phosphatidylcholine and lyso-phosphatidylethanolamine. The UPC2 has been applied to biological samples and showed successful separation of lipid classes. Separation and analysis of free fatty acids and neutral lipids was also developed. These results indicate that UPC2 is a promising chromatographic technique for FSV, carotenoids and lipids analyses.

Keywords: *Vitamins, Carotenoids, Lipids, SFC*

M-6
SCREENING HERBAL SUPPLEMENTS USING STATISTICAL MODELING TO FIND AND IDENTIFY ADULTERANTS AND CONTAMINANTS USING DIRECT ANALYSIS IN REAL TIME (DART) –MS.

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Introduction: The FDA Food Safety Modernization Act includes provisions for monitoring herbal supplement composition, mandating testing to ensure the presence and quality of active herbal ingredients while monitoring for potential contaminants and adulterants. Due to the active nature of substances involved and potential variability in formulation, batch-to-batch monitoring is desirable. Direct analysis in real time (DART)–MS enables a fast, inexpensive analysis and measurement of the substance of interest. A program designed to complete automated statistical analysis and comparison of samples against a custom model to identify the presence of the substance of interest, as well as the absence of contaminants and/or adulterants is described.
Methods: A set of commercial supplements (Black Cohosh) from capsules was prepared for analysis. To simulate contaminants, pesticide was added at several different concentration levels. The samples were then analyzed using DART–MS. Analyses of different aliquots of sample at increasing desorption gas temperatures. Resulting spectra were collected, and used to create a statistical model of spectra for both the supplement and supplement containing known contaminants. Subsequent tests were run to determine the effectiveness of the model.
Preliminary Data: Preliminary data from a set black cohosh capsules was collected; 30 samples for each of 3 tablets. The data was background-subtracted against a blank collected using the same method, and analyzed for anomalies using a set of heatmaps created using the R statistical package. After verification, the samples were imported into a statistical package (AnalyzeIQ Software from AnalyzeIQ Ltd) and used to create an SVM (support vector machine) model for the sample. The pesticide-spiked samples were then introduced, and the statistical model was used to determine that these samples were non-similar to black cohosh using a set of predefined statistical variables. The ability of the DART–MS method to simultaneously collect rich spectra of intact molecular ions allowed for the determination that the sample was contaminated. A model was then built for the pesticide dimethoate. The sample was run against this model to determine the presence of this pesticide. The workflow demonstrates the ability of the system to quickly and effectively screen for adulterants and contaminants within the herbal supplements market.

Keywords: *DART, Herbal Supplements*

M-7
UPLC–ELSD METOD FOR THE ROUTINE DETERMINATION OF GLUCOSAMINE, CHONDROITIN SULFATE AND HYALURONIC ACID IN JOINT SUPPLEMENTS

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Glucosamine (GSA), chondroitin sulfate (CS) and hyaluronic acid (HA) are carbohydrates, which are involved, together with collagen, in building cartilage where they are part of the proteoglycans. It has been shown, that consumption of these compounds can relieve the symptoms accompanying osteoarthritis and other joint diseases affecting millions of people worldwide. The latest studies are aimed to treat osteoarthritis, development of new medicines and joint supplements containing these substances. The increasing consumption, range and popularity of these supplements raise the necessity for a comprehensive assessment of their quality, safety and efficacy. Opposed to medicines, food supplements are not under such a strict legislative control in the Czech Republic, therefore doubts appear that these products really contain the mentioned ingredients in a quantity declared on the packaging. This study was focused on the development and optimization of simple and fast analytical procedure for the determination of HA, CS and GSA in joint food preparations. For identification and quantification of monitored compounds, the ultra-high performance liquid chromatography coupled with evaporative light scattering detector (UPLC–ELSD) was employed. The LC separation of CS and HA was performed on the Presto FF C18 column (150 mm × 4.6 mm I.D., 2 µm particle size, Impakt, USA). The mobile phase consisted of Mili-Q water (A) and acetonitrile (B) both with 5 mM trichlorocetic acid. For LC separation of GSA, Acquity UPLC® BEH Amide column (100 mm × 2.1 mm I.D., 1.7 µm particle size Waters, USA) and as the mobile phase, 80% acetonitrile (A) and 30% acetonitrile (B) both in Mili-Q water and 0.2% triethylamine, was used. These methods give good performance characteristics - recoveries and repeatabilities 95% and 5% for the GSA, 92% and 2% for CS and 87% and 6% for HA. Th e achieved instrumental quantification limit (IQLs) were 0,025; 0.1 and 0.025 mg/ml for GSA, CS and HA respectively. Following the successful method development and validation, the monitoring study of HA, CS and GSA content in 22 selected joint food supplements, available on the market in the Czech Republic, was performed.

Keywords: *Glucosamine, chondroitin sulfate, hyaluronic acid, food supplements, UPLC–ELSD*

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M-8
EFFECTS OF ANIMAL FAT SUBSTITUTION WITH RED PALM OIL ON THE PROPERTIES OF CHICKEN SAUSAGE

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Today's consumers focus on their health and body and they want to extend their life and improve it, the result of which is that they increasingly avoid foods containing cholesterol and saturated and trans fatty acids. Animal fat is an essential component in processed meat products such as sausages. However, animal fats are high in cholesterol and saturated fat contents. Therefore, substituting animal fat with palm oil which is rich in natural carotenes and vitamin E can be an alternative for meat products. The aim of this study was to replace animal fat with red palm oil (NVRO, NVRO 50 and NVRO 100) in chicken sausage and determine the properties of chicken sausage. Result showed that plam oil (NVRO) based chicken sausage had highest total carotenoids. Palm oil replacement had a significant effect on redness values of the samples. Replacement of animal fat with palm oil significantly increased MUFA, PUFA and MUFA + PUFA/SFA ratios. The chicken sausage cont ain palm oil (NVRO 50) had highest score in overall acceptability. The results indicated that palm oil has number of advantages over animal fat that it can improve nutritional value of end product.

Keywords: *Chicken sausage, animal fat, red palm oil, chicken sausage properties*

M-9
CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN COMMERCIALIZED ALGAE

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Algae have demonstrated an enormous range of applications, such as cosmetics, medicine, human nutrition, and biofuels, between others. Recently, algae have shown a potential for preventing diseases caused as a result of oxidative stress. For this reason, antioxidant activity is one of the most studied activities from these algae due to the interest of the health implications as preservatives and protectors against oxidation in food and cosmetics and also due to their potential as functional ingredients. In the present work, a comprehensive methodology was initially carried out for the screening of bioactive compound of algae commonly commercialized in Spain (Arame, Dulse, Hijiki, Nori, Agar, Wakame and Kombu), based on in vitro and LC–HRMS metabolomics studies. Therefore, the final objective was to demonstrate the usefulness of the method to perform screenings of different algae species in order to attain bioactive compounds, which could be used in the industry as functional ingredients, as well as for the authentication of these algae. With this aim, in vitro antioxidant studies were used as a first tool to evaluate potential bioactive compounds. Antioxidant activity of two different extraction solvents (aqueous and ethanolic) was evaluated by Trolox Equivalent Antioxidant Capacity (TEAC) procedure. Results confirmed that Arame ethanolic extract had higher antioxidant activity, however aqueous extract showed optimal antioxidant activity. On the other hand, the extracts were studied using UHPLC–Q–Exactive. These extracts contained thousands of compounds and their metabolomics fingerprinting was acquired by Full MS/ddMS2, allowing accurate resolving power MS and MS/MS data for marker identification. Data analysis was involved the use of SIEVE v2.1. In his research, Principal Compound Analysis (PCA) allowed Arame to be separated from the rest of algae The developed procedure allows estimating the functional activities of extracts obtained and even more important, to correlate these activities with their particular chemical composition. By applying this methodology it was possible to carry out the screening for bioactive compounds in seven commercialized algae.

Keywords: *Algae, metabolmics, bio-assay, bioactive compounds*

M-10
EFFECTS OF UV-B RADIATION ON COMPOSITIONAL PROFILE OF MUSHROOM POWDER USED AS DIETARY SUPPLEMENTS

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Mushrooms exposed to UV-B radiation produce elevated levels of vitamin D, and the dried powder is used as a dietary supplement to provide nutritional benefits. However, it is unknown if other chemicals (both beneficial and toxic) are also produced. Chemical composition changes of four types of mushroom powder dietary supplement exposed to different levels of UV-B irradiation were analyzed for the bioactive naturally occurring mushroom anti-oxidant, ergothioneine, other natural polyphenolic anti-oxidants: e.g. flavonoids, lignans, and others, and selected phytosterols. Four types of mushroom powder consisting of white, brown (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*) and shiitake (*Lentinula edodes*) mushrooms from three different treatment groups (control, low and high UV-B exposures) were analyzed. Phytosterols, including ergosterol, cycloartenol, β–sitosterol, campesterol, stigmasterol, brassicasterol, sitostanol (stigmastanol) and lupeol were detected as derivatized trimethylsylil ethers with gas chromatography triple quadrupole tandem mass spectrometry (GC–MS/MS) and quantified with cholesterol as an internal standard. Ergothioneine and multiple polyphenolic compounds were analyzed with liquid chromatography triple quadrupole tandem mass spectrometry (LC–MS/MS). Recovery experiments for all groups of chemicals were conducted for method evaluation, and the method for phytosterols was further validated with the use of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3251 Serenoa repens extract for selected phytosterols with certified values. Ergothioneine concentrations found in mushroom powder were 0.4–10.4 mg/g dry weight (dw), and were not affected by UV-B radiation. No polyphenolic compounds were detected. Ergosterol concentrations were 2.4–6.2 mg/g dw. As expected, ergosterol concentrations decreased with the increased level of UV-B treatment for all, but white mushrooms. Campesterol concentrations were 14–43 µg/g dw, and were not affected by the UV-B radiation. This study showed that UV-B radiation used to elevate vitamin D2 levels in mushrooms did not alter compositional changes of investigated bioactive compounds. These results suggest that mushroom powder dietary supplements not only provide vitamin D, but also may be a good source of phytosterols and the naturally occurring mushroom anti-oxidant ergothioneine.

Keywords: *Mushroom, dietary supplement, UV-B radiation*

ORGANIC CROPS AND FOODSTUFFS

(N-1 – N-5)

N-1
PEROXIDASE ACTIVITY AND MULTIPLE MOLECULAR FORMS IN BACTERIAL SPOT AND BACTERIAL CANCER DISEASED TOMATO

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Bacterial diseases of vegetables, including tomato plants cause serious damage, resulting in economic loss and human suffering. Among the bacterioses the most deleterious on tomatoes are bacterial spot caused by the bacterium *Xanthomonas vesicatoria* and bacterial cancer caused by Corynebacter michiganense. These diseases negatively affect plant development, harvest yield and quality. Little is known on influence of bacterial infection on plant metabolic activity; however it is extremely important for understanding of plant defense mechanism and development of a proper management strategy against the diseases, including biological means for production of safe, organic tomato. The aim of the work was to study plant response to bacterial infection at the level of oxidative enzymes, such as peroxidase and phenoloxidase. Healthy and diseased tomato plants were collected in different tomato production regions of Georgia in 2010–2011 years. Activities of peroxidase and phenoloxidase activities, as well as their molecular forms by means of polyacrylamide gel electropforesis (PAGE) have been studied in healthy and diseased by bacterial spot and bacterial cancer tomato plants and fruits at different stages of development. Significant increase in peroxidase and inhibition of phenoloxidase activities by spectrophotometric method in diseased tomato leaves and fruits were detected. The increase in peroxidase activity in diseased plants was manifested by the increase of enzyme activities bands on corresponding zymogram after PAGE. Besides, in addition to two molecular forms, four and seven new izoforms are formed in bacterial spot and bacterial cancer affected tomato leaves, respectively. As for tomato fruits, formation of three additional molecular forms of the enzyme was estableshed. The role of peroxidase in tomato defence mechanism in bacterial spot affected tomato is discussed.

Keywords: *Tomato bacterial spot, tomato bacterial cancer, Xanthomonas vesicatoria, Corynebacter michiganense, peroxidase*

N-2
DETERMINATIN OF THE MINERAL COMPOSITION (CA, FE, P, MG, K, NA) OF ORGANIC AND CONVENTIONAL GOAT'S AND EWE'S CHEESES USING NEAR INFRARED SPECTROSCOPY (NIRS)

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The mineral content of the milk depends on numerous factors, such as genetic characteristics, the stage of lactation, environmental conditions, type of pasture and soil contamination among others. The analysis of the mineral compounds in milk and cheeses is performed by inductively coupled plasma atomic emission spectrophotometry, ion exchange liquid chromatography, instrumental neutron activation analysis, atomic absorption spectrophotometry, atomic emission spectrophotometry and flame and graphite furnace atomic absorption spectrometry. In the last decades techniques like near infra-red (NIR) spectroscopy has been developed for the determination of majority parameters in cheese. Indeed, some studies on the prediction of the mineral compositions in fresh and freeze-dried cheeses or cheeses made from different percentages of milk (cow's, ewe's and goat's) have been carried out. In the present work we study the use of NIRS technology together with the use of a remote reflectance fibre-optic probe for the determination of mineral composition (Ca, Fe, P, Mg, K, Na) in forty two samples of organic and conventional cheeses. The cheeses were elaborated with milk from ewes and goats coming from different Spanish geographical areas (i.e Castilla y León, Castilla-La Mancha, Pais Vasco and Andalucia) that are characterized by different environmental conditions and type of pasture, and they ripened from two weeks up to 3 months. The NIR spectra were recorded in reflectance mode, applying the fibre-optic probe directly onto the cheese sample, with no sample preparation or manipulation. The mineral content of reference was determined by ICP-optic spectroscopy. The results of this work show that it is possible to rapidly quantify calcium, iron, phosphorus, magnesium, potassium and sodium in any of the cheeses by direct application of the fibre-optic probe on one slice of the sample without previous destruction or treatment of the sample. Calibrations were performed by modified partial least squares regression (MPLS). The multiple correlation coefficients (RSQ) showed values among 0.68 for magnesium and 0.92 for calcium and the prediction standard errors were lower than 10%. The prediction capacity of the model obtained was evaluated with the ratio performance deviation (RPD). If the RPD value is greater than 2.5 the model is considered suitable. The values obtained for RPD parameter that were 4 for calcium, 3.5 for sodium, 3.4 for phosphorus, 2.8 for potassium, 2.3 for iron and 1.9 for phosphorus. These results are even better than those previously reported for cheeses of with percentages (0–100%) of milk from different species (cow, ewe, goat). These results demonstrate that the capacity for prediction can be considered excellent for the Ca, Na, P and K, and acceptable for iron and magnesium.

Keywords: *Near infrared spectroscopy, mineral composition, organic, ewe's cheese, goat cheese.*

Acknowledgement: *The authors are grateful to University of Salamanca for funding the project.*

N-3
DIFFERENTIATION OF SPANISH ORGANIC AND NON ORGANIC GOAT'S CHEESES USING NEAR INFRARED SPECTROSCOPY IN COMBINATION WITH INTERVAL EXTENDED CANONICAL VARIATE ANALYSIS (IECVA): A PRELIMINARY STUDY

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Food traceability is aimed at protecting the consumer and food producers from fraud. The term organic production is used under diverse denominations such as biological and ecological. The organic manufacture of dairy products has increased in recent years. Near Infrared Spectroscopy (NIRS) provides fast, accurate, direct and non-destructive analysis. This technique coupled with chemometric tools could provide a method to undertake the traceability tasks. Interval extended canonical variate analysis (ECVA) is a recent chemometric classification tool representing a new approach for grouping samples based on the canonical variates analysis but extended to multivariate covariate data using an underlying PLS engine. The iECVA tool performs a series of ECV analyses, one for the whole spectrum and one for each defined interval (subregion of the spectrum having full resolution). At last, the performances of each interval are compared among each other and against the overall model. Thirty-one goat's cheese samples were collected from different Spanish geographical areas (i.e Badajoz, Burgos, Cádiz, León, Madrid, Tarragona and Zamora) and ripening time (up to 3 months). Eighteen samples were allocated into the organic set and the other 13 into the non organic set according to their production system. Cheese spectra were recorded with a Foss NIRSystem 5000 equipped with a fibre optic probe of remote reflectance covering the spectral range between 1100 and 2000 nm. In order to scrutinize the spectra for signals able to distinguish their production system (i.e organic or non organic), interval extended canonical variate analysis (iECVA) was carried out on the cheese spectra using 12 equally sized subintervals. The models were validated using the leave one out procedure. The iECVA analysis reveals that the 1328-1404 nm interval was able to reduce the number of misclassifications from 9 to 6 with respect to the global model, which represented a reduction about 33%. The number of PLS factors was also reduced from 5 to 4 with respect to the global model. The number of samples correctly classified using the proposed model was 81%. The procedure reported here seems to have potential for a fast and reasonably inexpensive analysis. Nevertheless, a comprehensive study using a large number of samples should be made. Furthermore, individual models for each geographical area should also be developed and tested in order to compare their accuracy with the global one.

Keywords: *Goat's cheese, organic, near infrared spectroscopy, chemometrics, interval extended canonical variate analysis*

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N-4
SPECIFIC BACTERIOPHAGE FOR SAFE TOMATO PRODUCTION

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Demand for organic, safe foods is driven by concerns for personal health and for the environment and is permanently increasing. *Lycopersicon esculentum* Mill (tomato) is an important vegetable crop that contains large amount of microelements and is rich in vitamins – A, B1, C, B9 and E. Licopene, vitamins C and E, and other tomato constituents prevent from forming of toxic oxygen radicals. Warious diseases, among which bacterial spot caused by the Gram-negative phytopathogenic bacterium *Xanthomonas campestris pv. vesicatoria* is the most devastating in Georgia and worldwide. The disease can cause severe losses in tomato crop yield and fruit quality, which is generally unmarketable and negatively affects the economics of the country. For instance, in George, Florida and the Caribbean this disease may cause decrease in tomato productivity up to 50%, while in some Canadian fields loss of productivity can reach 60%. There is tremendous demand in natural biocon trol of the disease and application of specific bacteriophage for this reason is prospective. The main advantage among numerous to phage therapy/decontamination the main advantages are that they are very specific to the disease-causing bacterium, naturally exist in environment, are self-replicating and self-limiting - they replicate only as long as the host bacterium is present in the environment, but are quickly degrading in its absence. They are non-toxic to any eukaryotic cells. But, their presence has been reported to reduce plant growth and reduce plant nitrogen content, in a couple of small-scale experiments (Ahamd and Morgan, 1994; Johnson, 1994). This effect, if true, could possibly be a consequence of phage antagonism against beneficial bacteria. The efficiency of 7x107p.f.u/ml bacteriophage, consisted of 3 pages lines isolated and purified in 2010–2012 in Georgia, were tested in greenhouse conditions on tomato fruits, artificially contaminated with 109c.f.u/ml c ulture of X. vesicatoria pathogenic strains. Spraying of artificially infected fruits with phage instantly or after 24 hours hindered initiation of the disease. The spray a week later stopped development of the disease. The activities of key nitrogen assimilation enzymes, such as glutamine synthetase and glutamate dehydrogenase were decreased by 15–20% in affected but not phage treated plants. Control level of malate dehydrognase, an important enzyme of Tricarboxylic acid cycle leading to energy generation was also maintained in phage treated plants. These data indicate on tomato normal growth and development under phage application.

Keywords: *Lycopersicon esculentum, Xanthomonas campestris pv. vesicatoria, tomato bacterial spot, specific bacteriophage*

N-5
INFLUENCE OF GROWING CONDITIONS ON SELECTED PARAMETERS OF SPELT FLOURS

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Complex analysis of the influence of growing conditions on selected parameters of Hungarian wholemeal spelt flours prepared from two spelt varieties ÖKO-10 and Lajta grown under organic and conventional production practices in 3 different Hungarian regions (Mosonmagyaróvár, Rábcakapi, Sopronkövesd) and harvested in different year (2011 and 2012) was performed. Solid flour samples were analyzed to ash, dry matter and total nitrogen content but also to minerals and trace elements content was evaluated by AAS. Besides that, UV/Vis/NIR spectral and CIE L*a*b colour quality attributes were evaluated. Methods of EPR and UV/Vis spectroscopy was involved to determine total polyphenols (TPC) and total flavonoids (TFC) content as well as some other characteristics of antioxidant properties (•DPPH/ABTS•+ radical-scavenging ability) of flour extracts in 50% ethanol/water solution (v/v) which was selected as the most promising extraction system from extraction y ields point of view. Moreover, amino acids profile and total saccharides content were determined by HPLC–MS and HPLC-RID system, respectively. The whole dataset of experimental characteristics was processed by ANOVA Tukey-HSD and multivariate statistical methods, employing the methods of principal component factoring (PCF) and canonical discrimination analysis (CDA) in order to assess the influence of various factors, i.e., year of production, way of farming, spelt varieties and geographical locality on the monitored characteristics of spelt flours.. As follows from the obtained results, majority of determined experimental characteristics is significantly influenced by all the above-mentioned parameters. Both, organic and conventional flours from 2011 harvest exhibited the higher lightness, whiteness, flour colour index, as well as total amino acids and total saccharides contents, while these from 2012 were characterised by higher nitrogen, dry matter, total polyphenols and total flavonoids content and •DPPH/ABTS•+ radical-scavenging activity. The obtained results clearly confirmed that methods of multivariate statistical analysis represent an efficient tool capable of spelt flours differentiation according to the parameters described above. ANOVA and PCF proved also that all the experimental characteristics revealed their importance for the discrimination of the spelt flours. CDA, in dependence on the selected discrimination criterion, possessed very high recognition scores. Recognition of spelt flours according to the year of production reached 100% correct classification, while in case of spelt variety differentiation the recognition score of approx. 70% was obtained, confirming thus great heterogeneity and variability of the results.

Keywords: *Spelt flours, growing conditions, composition analysis, multivariate statistics*
Acknowledgement: *This contribution is the result of the project HUSK – 0901/1.2.1/0010 "Utilization of regional sources for functional food production" realization, supported by European Regional Development Fund within the Hungary-Slovakia 2007-2013 Cross-border Cooperation Programme. This contribution is also of the result of the project ITMS 26240220050 „A strategy of acrylamide elimination" implementation, supported by the Research & Development Operational Programme funded by the ERDF.*

PACKAGING CONTAMINANTS

(O-1 – O-19)

O-1
DETERMINATION OF EPICHLOROHYDRIN IN EPOXY LACQUER USED IN CANS FOR FOOD AND BEVERAGE BY GC–MS

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Epichlorohydrin (1-chloro-2,3-epoxypropane; C_3H_5ClO; CAS RN 106-89-8) is an epoxide which is highly reactive and is used in the production of a variety of industrial chemical products. The main use of epichlorohydrin is in production of epoxy resins, which, among other applications, are used as thin protective coatings inside food and beverage cans. Epoxy resins can contain residual concentrations of epichlorohydrin and trace levels may be present in the manufactured can coating. In this situation migration of epichlorohydrin into the foodstuff can occur. Epichlorohydrin is classified as probably carcinogenic to humans and on this basis exposure is considered harmful at any levels. The European Committee for Standardization has established a technical specification which describes a chemical analytical method for determination of residual content of epichlorohydrin in coatings (CEN/TS 13130-20:2005). The principle of the method includes organic extraction of the sample material, microdestillation of the extract, derivatization of the epoxide and reversed phase HPLC with fluorescence detection. This method has previously been set up in our laboratory and although the performance was satisfactory the procedure appeared too complex and labour intensive. Therefore, a simpler analytical method based on GC–MS was developed before an official control program on samples of food contact materials was carried out. The new analytical principle is as follows: the can with epoxy lacquer is cut into squares of approximately 1–2 cm^2. A number of these squares with a total surface area of 1 dm2 are extracted for 6 hours with 20 ml of acetonitrile. The extract is injected (1 µl splitless at 120°C) on a gas chromatography column (CP WAX 52B, 30 m × 0.25 mm, DF=0.25 µm) and epichlorohydrin is detected by a quadrupole mass spectrometer with electron impact ionisation and selected ion monitoring. The quantification ion is at *m/z* 57 and three verification ions are monitored at *m/z* 49, 62 and 51 (in order of decreasing intensity). Quantification is achieved by external calibration. The method was validated by an experimental design which incorporated three analytical series where samples were spiked at three concentration levels and were analysed in quadruple measurements. The series were spread over several weeks and with the use of two technicians. Ruggedness testing was partly done by a statistical factorial design which reduced the required number of analyses. The tested parameters included extraction time, extraction temperature, GC injector temperature, GC to MS interface temperature and shelf life of the standard solutions. The developed analytical method has a limit of detection (LOD) of 0.08 µg/dm^2 and internal reproducibility of 8% RSD below 0.9 µg/dm^2 and 4% RSD above. Recoveries during the validation series varied from 88% to 114% with an average of 102%.

Keywords: *Epichlorohydrin, food contact materials, epoxy lacquer, can coating, method validation*

O-2
ANALYSIS OF ADDITIVES IN BIODEGRADABLE POLYMERS USING DIRECT SAMPLING ANALYSIS (DSA) TIME-OF-FLIGHT MASS SPECTROMETRY

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Biodegradable polymers are increasingly being used as an alternate to conventional commercial polymers because they provide an additional end of life scenario route. Biodegradable polymers can be made from renewable resources such as starch, chitin and cellulose or non-renewable resources such as poly(butylene adipate terephthalate). Additives are added to biodegradable polymers to increase their properties such as toughness, flexibility, or barrier properties thereby improving their functional capability. These additives can leach into their surroundings affecting the product in contact with them, such as food. The aim of this study was to use ambient ionization mass spectrometry to rapidly identify a wide range of additives in different types of biodegradable polymers. Commercially available salad containers made of poly(L-lactic acid) (PLLA) were examined for additives. Also, PLLA containing known additives including butylated hydroxytoluene (BHT), 0.95%alpha-tocopherol and poly(butylene adipate-co-terephthalate) containing 0.5% BHT, were examined by DSA

Keywords: *DSA, Biopolymers, Additives, Packaging*

O-3
QUANTITATIVE SCREENING OF POSSIBLE MIGRANTS FROM PAPERBOARD PACKAGING MATERIAL BY SOLID-PHASE MICRO EXTRACTION COUPLED TO GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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The reported method was developed for the determination of possible migrants from paperboard packaging material by usage of solid phase micro extraction and gas chromatography coupled to mass spectrometry. Different commercial SPME fibers and other parameters affecting the performance of extraction process including extraction temperature, extraction time, sample volume, salting-out effect, composition of extraction solvent and an additional sample pretreatment step were investigated during method development. Composition of extraction solvent together with SPME fiber coating and extraction temperature were identified as major influences and most critical parameters for the method. The best results were reached by application of the optimized SPME method applying Polydimethylsiloxane (PDMS) fiber, placing 0.5 g of paperboard sample directly in to head-space vial together with 8 ml of extraction solvent containing water and methanol. The final method was validated as a quantitative screening method according to the Commission Directive 2002/657/EC for group of important contaminants covering the representatives of phthalates, photoinitiators, phenols, and off-flavors deriving from the degradation of paperboard components including printing, coating and adhesives. For quantitation the standard addition procedure was employed. Detection limits ranged from 0.001 to 1.000 mg/kg and relative standard deviation was below 20% for all analytes. The final method was applied in a small survey covering paperboard samples of various quality including as virgin as recycled paperboard.

Keywords: *Packaging – migrants, paperboard, solid phase micro extraction, gas chromatography – mass spectrometry*

O-4
METHODS FOR IDENTIFICATION OF KNOWN AND UNKNOWN FOOD CONTACT MATERIALS BY MEANS OF HPLC–HIGH RESOLUTION-ACCURATE MASS SPECTROMETRY

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Packaging materials has recently been recognised as potential source of food contamination and got one of the most intensively studied compound classes nowadays. All food packaging materials can release amounts of their chemical constituents when they get in contact with food and any substance that migrates from the packaging into the food possessing potential health risk to the consumer. The amounts migrating from packaging into the food is may be even 100 times greater than the contribution of pesticides or other well known environmental pollutants. The analysis of packaging materials migrating into food is difficult based on the complexity and diversity of the physicochemical properties of these chemicals. Analytical methods shall be capable to identify and quantify as wide range of target compounds as possible not just at low concentration levels but also guarantee confirmation of the target analytes to prevent false positive or negative results. In addition to that, identification of new, up to now unknown migrating compounds is exponentially gaining on importance. In recent years GC based measurements provided numbers of information for volatile and low molecular weight substances, however to date, HPLC and high resolution accurate mass applications (HRAM–MS) have provided relatively little information on the more polar and non-volatile substances that may migrate into the food. The aim of our poster is to demonstrate strategies for identification of known as well as unknown compounds using LC based technology coupled to Orbitrap HRAM–MS technology. Strategies and practical examples with paperboard extracts will be demonstrated how targeted and untargeted screening methods can be successfully applied for identification or quantification of polar-apolar and low volatile packaging compounds. The high resolution mass spectra data (100k) with the latest software support tools will be demonstrated for getting high confidence about the results and minimize the number of falsely identified compounds.

Keywords: *Packaging materials, HR–MS, Orbitrap, unknown screening*

O-5
SCREENING AND CONFIRMATION FOR 35 PHOTOINITIATORS IN FOOD PROCUCTS BY LC–MS/MS USING TRIGGERED MRM ACQUISITION AND LIBRARY COMPARISON

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There is an increasing concern about contaminants in food products. This does include compounds like plasticizers or printing ink ingredients which are introduced in the food from the packaging materials. While printing inks are typically only applied to the external surface of the food packaging, they might migrate into the food or accidentally contaminate the food by the set-off process, when printing ink ingredients like photoinitiators can be transferred unintentionally from the printed side of the packaging to the food contact surface. Photoinitiators can be analyzed by GC–MS or LC–MS. There are not yet many multi-photoinitiator methods published although modern analytical instruments are capable in analyzing an increasing number of compounds with a single injection. In this presentation we show the application of ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) for the analysis of 35 photoinitiators in food. A method was set up using the triggered multiple reaction monitoring (TMRM) acquisition mode with two primary transitions and up to 6 confirmatory ions per compound which were acquired data dependently. A variety of food matrices was extracted and analyzed using this method. For method validation an extract of dried pasta was spiked at 3 different levels with a mixture containing all target compounds. Confirmation of the target compounds was done by comparing the qualifier/quantifier ratios and by a scoring based on the comparison of the TMRM spectra with a TMRM library containing spectra for all analyzed photoinitiators. A method for the analysis of 35 photoinitiators using TMRM acquisition has been developed and successfully validated for pasta samples. In addition a library was created containing the product ion spectra of all target compounds. Limit of quantitation for the majority of compounds was below 1 ng/g in the food matrix. The comparison of the TMRM method acquiring up to 8 transitions per compound with a conventional method acquiring only two transitions per compound showed good agreement in terms of sensitivity, linearity, and peak areas for the quantifier ions. Excellent precision data for replicate injections show that quantitation has not been compromised when triggering additional transitions. In addition full product ion spectra acquired even at the lowest concentration levels allowed the unequivocal identification of the target compounds even in complex matrices and several examples thereof are shown.

Keywords: *Photoinitiators, packaging contaminants, LC–MS/MS, triggered MRM*

O-6
RISK ANALYSIS OF FOOD CONTAMINATION BY LEAD FROM GLASS PACKAGING

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Common primary raw materials used for production of glass packaging contain heavy metals only in amounts that do not cause exceeding of the specified maximum concentration limits. The reason why heavy metal concentrations increase is the fact that recycled glass contaminated by these heavy metals is used. The main contaminating products are television tubes, fluorescent tubes and glass electronic components. Current chunk glass recycling technologies do not allow for effective separation of shards containing high levels of heavy metals and these contaminated shards then constitute the by far largest contaminating factor of glass packaging. The legal regulations related to packaging and packaging waste Act 477/2001 Coll. on packaging and Directive 94/62/EC (which specify the minimum amount of recycled materials which must be used during the production process) are important factors leading to the current situation. The test results show that with glass that i s produced shortly before the migration tests (i.e. its functional surface is not disrupted by corrosion), the lead does not migrate into the simulator and food in a concentration greater than the legally permitted value even in cases when the concentration of lead in the glass material exceeds the permissive values 5 times. The same is true for the tests carried out under higher temperatures (90±5)°C, for tests carried out on samples with surface renewed by action of acids including combination of both these test modifications. From this viewpoint, there is no risk of contamination of the filling by lead.

Keywords: *Lead, glass, risk analysis, food contamination*

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O-7
SINGLE-STEP EXTRACTION AND CLEANUP OF BISPHENOL A IN SOFT DRINKS BY HEMIMICELLAR MAGNETIC SPE PRIOR TO LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

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Bisphenol A (BPA) is a high-volume production chemical. Until recently, it had not been considered a chemical of high concern because traditionally it has been counted as a weak estrogen. However, a large number of recent in vitro studies have shown that the effects of BPA are mediated by both genomic and non genomic estrogen-response mechanisms, with the disruption of the cell function occurring at doses as low as 0.23 ng L^{-1}. BPA is mainly used in the manufacture of polycarbonate plastic and epoxy resins. These manufactured materials are extensively used as food containers and food can linings. Heat and either acid or basic conditions accelerate the hydrolysis of the ester bond linking BPA monomers and lead to BPA release and migration into the food. Intake of BPA-contaminated foods constitutes the primary route of human exposure to this chemical. Concentrations reported for BPA range in the interval 0.1–3.4 ng mL^{-1} and 0.3–458 ng g^{-1} for drink and food, r espectively. Estimated intakes of BPA for humans are all well below the Tolerable Daily Intake of 0.05 mg/kg body weight/day set by the European Food Safety Authority (EFSA) for this substance in 2006. However, new findings from ongoing studies on low dose effects observed in rodents have urged EFSA to launch a full re-evaluation on BPA. This reassessment of human exposure to BPA makes it necessary to take into account all the possible dietary sources and consequently, to analyze a vast array of products and brands. In this context, it is recognized the need for development of simple, high-throughput analytical methods for the determination of BPA in high consumption, low BPA-content food such as drinks. Only a few methods are sensitive and selective enough to give accurate data of BPA levels in soft drinks and mineral water. Sample preparation still constitutes the key-step for the determination of BPA in drinks and it is the origin of the main drawbacks in the available methodologies. Solvent extraction and solid phase extraction are by far the most used extraction techniques for both isolation of BPA and clean-up of matrix components. In the present study, hemimicelles of tetradecanoate chemisorbed onto magnetic nanoparticles are proposed as a sorbent for the single-step extraction and cleanup of BPA in soft drinks. This sample treatment, combined with LC separation and electrospray ionization tandem mass spectrometric detection, provides a sensitive and reliable method for the determination of BPA in drinks. The limit of quantitation obtained for the method, 0.03 ng mL^{-1}, was below the usual range of concentrations reported for BPA in soft drinks. The proposed method was successfully applied to the determination of BPA in different samples acquired from various supermarkets in southern Spain; the concentrations found ranged from 0.066 to 1.08 ng mL^{-1}. Recoveries from samples spiked with 0.33 ng mL^{-1} of BPA ranged from 91 to 105% with relative standard deviations from 3 to 8%.

Keywords: *Bisphenol A, hemimicellar magnetic SPE, soft drink, LC–MS*

O-8
IS MINERAL OIL CONTAMINATION REALLY MINERAL OIL? DETAILED ELUCIDATION BY USING COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH DUAL DETECTION

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Mineral oil is a quite widespread contaminant of foods. It derives from different sources, but more recently attention has been focused mainly on paperboard packaging. Such a contamination derives from the offset printing inks applied directly to the packaging, and/or from the ink present in the recycled fibers used (generally deriving from newspapers). Mineral oil contains proportions of mineral oil saturated hydrocarbons (MOSH, including n-alkanes, isoalkanes and cycloalkanes), and mineral oil aromatic hydrocarbons (MOAH), which both are characterized by humps of unresolved complex mixtures (UCM) after gas chromatographic (GC) analysis. Resolution can be greatly improved using a comprehensive multidimensional GC (GC×GC) approach, which allows to define a more detailed profile of the compound distribution in the unresolved hump, especially for the MOAH fraction, where compounds can be separated according to the number of rings. Despite the lack of structural information (obtainable using a mass spectrometer – MS - detector), the flame ionization detector (FID) is the detector of choice to reliably quantify such humps, because FIDs provide virtually the same response per unit of mass of hydrocarbons, on the contrary of the MS detector. Moreover the lack of a proper calibration standard is a serious quantification problem in MS analysis. The aim of the present work is to develop a method to simultaneously quantify and confirm mineral oil contamination, by using GC×GC with dual detection: FID for quantification purposes, and MS for confirmation. The MOSH and MOAH fraction were separated by using an SPE cartridge, manually packed with silvered silica gel (Ag-SPE), before injection in the GC×GC system. The presence of interfering compounds, in the fraction of interest, can be determined both by the position in the bidimensional plot and the mass spectrum profile acquired simultaneously. The quantitative results (both for the MOSH and the MOAH fractions) obtained were compared with those derived by performing large volume injection (LVI) GC–FID, after the same Ag-SPE fractionation step, and through a hyphenated liquid-gas chromatographic system (LC–GC).

Keywords: *Comprehensive gas chromatography (GC×GC), dual detection, mineral oil, mineral oil saturated hydrocarbons (MOSH), mineral oil aromatic hydrocarbons (MOAH)*

O-9
LOW LEVELS OF ALUMINIUM IN SOFT-DRINKS ON FINNISH MARKET

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High aluminium (Al) intake may have a risk for human health. The major route of exposure to aluminium for general population is through food. It is typically found from bread, cakes, some vegetables and so forth. WHO/FAO has set a PTWI value of 1 mg.kg^{-1} body weight per week and later on EFSA set a TWI of 1 mg kg-1 body weight per week [1]. The maximum limit has been set only to water and wine, maximum limits being 200 µg.l^{-1} and 8000 µg.l^{-1}, respectively. Typically Al levels in soft-drinks and beer are lower than 200 µg.l^{-1} [2]. The levels of Al were analyzed from (n=37) canned Organic Cola, Coca-Cola, Finnish Rio Cola and other soft drinks. Samples were diluted to 1:25 in 2% (*v/v*) HNO_3 solutions with internal standard of 69Ga/89Y and analyzed by using ICP–MS instrumentation (Thermo Fisher Scientific XSeries II, ICP–MS). NIST CRM Lake Ontario Water (33.6±4.5) µg.l^{-1} and NIST CRM Thames River Water (76±13) µg.l^{-1} was used as a reference material. The fully validated and accredited method has a measurement uncertainty of 24%, with a LOD of 0.5 µg.l^{-1} and a LOQ of 1.0 µg.l^{-1} were determined. We analysed 37 samples which varied from Coca-Cola to organic cranberry juices. In these samples the level of Al varied from 54 µg l^{-1} to 514 µg.l^{-1} and the medium of all samples was 201 µg.l^{-1}. The highest level of aluminium was detected in cranberry juice (514 µg.l^{-1}, n=1). The storage of samples in open cans (sealed with laboratory Parafilm “M”) seem to increase the aluminium levels as high as 13,540 µg.l^{-1} in a single sample of organic Cola. Our data is in line with the data from Sweden in which the Al in soft-drinks were found to vary from 90 to 130 µg.l^{-1}. Also in Switzerland levels of Al in Coca-Cola was reported to vary from 14 to 250 µg.l^{-1} and in diet Coca-Cola from 46 to 170 µg.l^{-1} [2, 3]. If we estimate the aluminium exposure from soft drinks with a 0.1% bioavailability, one needs to consume 1200 l of Cola or 300 l other soft drinks to get an intake of TWI 1 mg kg^{-1} body weight per week.

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Keywords: *Aluminium, commercial sof-drinks, accredited ICP-MS method*

O-10
NEW STANDARDS FOR PAPS – EMERGING FLUORINATED SURFACTANTS IN FOOD CONTACT MATERIALS

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Polyfluoroalkyl phosphate surfactants (PAPS) are a type of fluorinated chemicals that are used to grease and water proof food wrappers. The substances are applied to food contact paper as coating because they prevent oil soaking into paper from fatty food. A study in 2005 showed that PAPS and similar compounds used in these applications can leach from microwave-popcorn packaging into the food. Another study by the University of Toronto chemists in 2007 showed that once ingested, PAPS are bioavailable and can be metabolized to form PFOA and other perfluorinated chemicals (PFCs). PAPS and other fluorinated surfactants in food contact materials therefore seem to be a significant source of exposure of PFC to both humans and the environment. The European Commission recommended the monitoring of perfluoroalkylated compounds in food of plant and animal origin to enable an accurate estimation of exposure. Especially perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), their precursors and PAPS in particular need to be monitored in order to estimate the relevance of their presence in food. However, the lack of commercially available standards of PAPS is making it difficult to determine the exposure of humans to these compounds. A project for synthesis of standards for PAPS has been established at CHIRON and a series of fluorotelomer alcohol substituted phosphate surfactants, both native and duterated such as 8:2 monoPAPS, di-PAPS and tri-PAPS, has been synthesized and analyzed.

Keywords: *PAPS, PFC, PFOA, labelled PAPS, standards*

O-11
DEVELOPMENT AND VALIDATION OF METHODS FOR THE DETERMINATION OF BENZOPHENONE AND 4-HYDROXYBENZOPHENONE IN FOOD PACKAGING AND BISCUIT

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Benzophenone and its derivatives, including 4-hydroxybenzophenone, as photoinitiators are used in UV printing. Photoinitiators are catalysts in the reaction of photopolymerization and are used during the UV curing process to promote fixation of printing inks onto packaging materials. While no residue of organic solvents from such printed materials can contaminate the food, results reported in the literature indicate that ingredients in UV printing inks are not always fully integrated into the polymer matrix and may migrate onto foodstuffs. Printing inks are not covered by specific European legislation, however, the Scientific Committee on Food (SCF) established a maximum of 0.6mg per kilogram of food as specific migration limit for BPs (Directive 2002/72/EC) and a tolerable daily intake of 0.01 mg per kg body weight. The aim of this study was to develop and validate methods for the determination of benzophenone and 4-hydroxybenzophenone in food packaging (cardboard and foil) as well as methods for the determination of benzophenone and 4-hydroxybenzophenone in biscuits by GC–MS. Packaging samples (cardboard and foil) were extracted 3 hours with dichloromethane and analyzed by GC–MS. Biscuits samples were extracted with a mixture of dichloromethane:acetonitrile (1:1, *v/v*) and centrifuged at 3000 rev/min. The supernatant was evaporated in stream of air, dissolved in hexane and extracted with acetonitrile. Acetonitrile layer was matched, reconstituted in dichloromethane and finally analyzed by GC–MS. HP-5MS 5% Phenyl methyl siloxane column was used for separation at a constant pressure in SIM mode. As an internal standard was used 4-hydroxy-4`-fluorobenzofenon. For method validation, the following parameters were evaluated, at two different spiked levels. The results were ranging between the following values: recovery from 81% to 109% for packaging and from 84% to 104% for biscuits; LOQ is 0.09 mg/dm² for packaging and 0.03mg/kg for biscuit; precision is 7.8% for packaging and 9.3% for biscuits; expanded measurement uncertainty of 7.5% and 8.3%, for the packaging and biscuits, respecitively. The correlation coefficient of the analytical curve were 0.99, for bensophenon and same for the 4- hydroxybenzophenon. This method was applied to 44 commercial samples, 40 samples of packaging and 4 samples of biscuits. In 7 samples of cardboard packaging content determined benzophenone in concentrations of 0.6 to 1.6 mg/dm^2, while in other samples the content was below LOQ. 4-hydroxybenzophenone was not detected in any samples.

Keywords: *Benzophenone, 4-hydroxybenzophenone, food packaging, biscuit*

O-12
MONITORING OF REMNANTS OF PACKAGING MATERIAL IN FORMER FOOD PRODUCTS

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The current high standards for food production imply that certain food products are declared unfit for human consumption. This situation can be due to problems of manufacturing, over-production, packaging defects, limited shelf life, etc. Nevertheless, these so-called former food products can be used for other high-value purposes, such as animal feeding. The usual requirements of quality and safety for these products have to be assured. The use of former food products is very important for a sustainable production of food and feed. Both bakery products and candy syrup are valued sources of nutrition for animal feeding. However, residues of packaging materials can still be present after unpacking and processing. Monitoring with methods for screening and quantification is necessary in order to assure a safe application of these products as feed ingredient. The method for bakery products is based on sieving the sample material, manual selection of particles that match the types of packaging materials (paper, board, plastic, clips, wires, etc.) from the appropriate fractions, defatting and dehydration of the selected material, weighing, and calculation of the percentage (w:w). The method is validated with values for selectivity, sensitivity, precision (recovery), robustness and level of quantification have been established. Candy syrup contains an approx. level of sugars of 65%. A part of these carbohydrates might be caramelised. The method for detecting packaging materials is focusing on dissolving the sugar fraction in hot water and on a subsequent automatic selection of the particles of packaging materials. The method is established, but not currently validated. In a period of six years 160 samples of bakery products were investigated in the Netherlands. The annual average levels of residues of packaging materials ranged from 0.03% to 0.06% (*w/w*). Assuming a tolerance limit of 0.15% (*w/w*), only 7 out of those 160 samples (4.4%) exceeded this limit. Until now, monitoring of candy syrup was not part of the Dutch monitoring program. The detection of packaging materials is an example of monitoring of physical contaminants. Although chemical contaminants attracts most of the interest in food safety control, legislation includes a range of limitation for physical contaminants. It is important to pay relevant attention to this group of hazards.

Keywords: *Former food products, packaging materials, physical contaminants, sustainability*

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O-13
EVALUATION OF THE MIGRATION OF 14 PHOTO-INITIATORS FROM CARDBOARD PACKAGING INTO TENAX® AND CEREALS USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY (UPLC–MS/MS)

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Mixtures of photo-initiators are commonly used in the formulation of inks, particularly UV-cured inks. After exposure to light, the photo-initiator decomposes into free radicals triggering the polymerization reaction, which causes the ink to cure onto the substrate. Consequently, drying times are much shorter for UV-cured inks than those with conventional solvent or water based coatings. Several alerts for food contamination caused by UV-curable inks occurred in the past. In 2005, Italian authorities withdrew thirty million liters of infant milk from the market due to the presence of the photo-initiator 2-Isopropylthioxanthone (ITX). Since then, other photo-initiators have also been found in food, such as 2-ethylhexyl-4-dimethylaminobenzoate in beverages and benzophenone in breakfast cereals. Evaluation of the migration of photo-initiators in food is very challenging due to the complexity of the matrix and the wide variety of foods that need to be analysed. Therefore, migration studies can be carried out using food simulants. The official simulant for dry food is poly(2,6-diphenylphenylene oxide), also known under its commercial name Tenax®. Valuable methods for the determination of photo-initiators in Tenax® are not numerous and do not use more than various photo-initiators in the same method. In this contribution, a fast and reliable confirmation method for the determination of 14 photo-initiators in Tenax® is presented. Migration experiments were performed by exposing carboard samples to spiked Tenax® for 10 days at 60°C. After migration the Tenax® was extracted and extracts were analysed using a UPLCTM system in combination with a XevoTM TQ-S mass spectrometer with electrospray ionization in positive mode (ESI+). In parallel, a confirmation method for the same 14 photo-initiators in food (cereals) was developed. Cereals samples were contaminated (spike), extracted and analyzed via the same UPLC-MS/MS method as used for the Tenax® experiments. A full in-house validation was done for both methods at the following levels: 0.25 µg g^{-1}, 0.625 µg g^{-1}, 1.25 µg g^{-1} and 2.5 µg g^{-1} Tenax® and 0.01 µg g^{-1}, 0.025 µg g^{-1}, 0.05 µg g^{-1} and 0.1 µg g^{-1} food. Because a matrix effect occurred for both methods, linear matrix-matched calibration curves (R^2 > 0.97), using an internal standard, were used for the quantification. Moreover both methods were found to be specific and precise, yielding in appropriate recoveries. Also very low detection limits ranging from 0.0125 µg g^{-1} to 0.125 µg g^{-1} Tenax® and 0.0005 µg g-1 to 0.01 µg g^{-1} cereals were achieved. Finally the presented methods were applied to real samples found on the Belgian market. It can be concluded that two fast and reliable methods were developed, that are able to confirm and quantify the migration of photo-initiators from the packaging into Tenax® and into cereals, showing their suitability for routine analysis.

Keywords: *Migration, photo-initiators, dry food, Tenax, confirmation*

O-14
A STUDY ON DIFFERENT STORAGE CONDITIONS AFFECTING MINERAL OILS MIGRATION FROM PACKAGING TO SEMOLINA AND EGG PASTA

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Most foodstuffs are provided with a packaging which carries out several important functions. However, the transfer of undesirable compounds can occur during the shelf-life of the product. In particular, cardboard packaging represents an important source of food contamination with mineral oil, when recycled fibers or mineral oil based printing inks are used. In this work MOSH (mineral oil saturated hydrocarbons), MOAH (mineral oil aromatic hydrocarbons), POSH (polyolefin oligomeric saturated hydrocarbons), and DIPN (diisopropyl naphtalenes) migration from packaging to dry foods has been monitored up to 1 year, focusing on the influence of food packaging material, fat content of the food, time and storage condition. Furthermore, contribution of hot melt adhesives used to close boxes to the total contamination was also evaluated. Semolina and egg pasta, of the same small size, were packed in plastic film bags and in recycled and virgin paperboard boxes and stored under two different conditions at ambient temperature. Same samples were stored on shelves to simulate the real common storage conditions, while others were wrapped in aluminum in order to force the migration only towards pasta, excluding any external influence. The mineral oil migration from a transport box consisting of corrugated board, through the primary packaging, was also evaluated. Migration behavior was studied measuring both the mineral oil amount lost by the packaging (calculated as the difference between pre- and post-contact contamination) and the mineral oil amount migrated in pasta samples after the exposure (subtracted from the pasta contamination at time zero). Diffusion of migrated mineral oil inside the product was also monitored by applying selective extraction methods (for semolina pasta). A POSH contamination was evident in pasta samples stored in plastic film. Very low contamination levels (< 0.6 mg/kg of MOSH) were found in pasta sample packaged in virgin paper. An important contribution due to the use of hot melt adhesives was evidenced. Higher contamination levels (about 5 and 12 mg/kg of MOSH for semolina and egg pasta, respectively) were found in pasta packaged in recycled paperboard for 1 year. Samples reached a steady contamination level, already after the first 1–3 months of storage, corresponding to about 20 and 50% of potential migration for semolina and egg pasta, respectively. The contribution of the external ambient was well evident in samples stored on the shelves (especially for egg pasta), while a little contribution due to the corrugated cardboard used as secondary packaging was observed.

Keywords: *Mineral oil, migration, recycled paperboard, pasta*

O-15
MULTI-RESIDUE ANALYSIS OF BISPHENOLS AND THEIR DIGLYCIDYL ETHERS IN CANNED FOODS FOR EVALUATION OF HUMAN EXPOSURE

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A reliable health risk assessment of bisphenols and their diglycidyl derivatives relies basically on its unambiguous identification and accurate quantification in the food chain. Most methods developed so far deal with the determination of BPA, bisphenols or diglycidyl derivatives, but no methods have been developed for the simultaneous determination of the whole of these target compounds. Multiresidue methods give a more realistic intake of bisphenols and permit labs to quantify them while saving costs and analysis time. The analysis of bisphenols and their derivatives in food requires very sensitive and selective instrumental techniques owing to their trace contents in foodstuff as well as sample matrix complexity. Even though the specific migration limit as set by the European Commission are much higher, there has been an increased quest for methods with very low limit of detection in order to asses human risk exposure at such levels. In this work, the development, optimization and validation of a multi-residue method for the determination of bisphenols [i.e bisphenol A (BPA), Bisphenol B (BPB), Bisphenol F (BPF), Bisphenol E (BPE)] and their derivatives [i.e bisphenol F diglycidyl ether (BFDGE), bisphenol A diglycidyl ether (BADGE), BADGE.2H_2O, BADGE.H_2O, BADGE.HCl.H_2O, BADGE.HCl, BADGE.2HCl and BFDGE.2HCl] in canned food is reported. The method was based on the single step microextraction and cleanup of the target contaminants with a supramolecular solvent made up of inverse aggregates of tetradecanol followed by liquid chromatography analysis with fluorescent detection. Chromatographic separation of all target compounds was achieved with baseline separation (Rs ≥ 1.52). The method detection and quantification limits ranged between 0.48–40.6 μg kg^{-1} and 1.17–97.5 μg kg^{-1} which are far below the migration limits set by the European Union. Method validation was carried out according the recommendatio ns of the European Commission Decision (2002/657/EC). Accuracy at 2 times the method quantification limits (MQLs) ranged between 82 and 107%; the repeatability and within-laboratory reproducibility at 5 times the MQLs ranged between 1.8–7.2% RSD and 4.6–9.9 %RSD respectively. The selectivity was high enough to allow the quantification of all the target contaminants using external calibration. Fourteen canned food samples including fruit, vegetable, meat and fish, all of them from local supermarkets in Córdoba (Spain), were analyzed, both bisphenols and their derivatives were found in the samples analysed at concentrations in the range 0.01–0.95 mg kg^{-1}. Only BPF and one of the isomers of BFDGE and BFDGE.2HCl were undetected. Recoveries in samples were in the range 70–110% .The performances of the proposed method meets the European Commission Decision criteria to be used as a generalized method to control the presence of bisphenols and their derivatives in cann ed food.

Keywords: *Supramolecular solvents, bisphenols, bisphenol diglycidyl ether, canned food, microextraction*

O-16
IDENTIFICATION OF POTENTIAL MIGRANTS FROM PRINTING INKS USED ON PAPER AND BOARD FOOD CONTACT MATERIALS

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Printing inks, used on paper and board food packaging, are often fairly complex formulations of colorants, resins, solvents, additives and photoinitiators. Although European legislation does not specifically cover printing inks in their supplied form, there are some legislative instruments which have an impact on materials and articles intended for direct contact with food, such as Article 3 of EU Regulation 1935/2004, that states that materials and articles intended to come into contact with foodstuffs shall not, in accordance with good shall be manufactured in accordance with good manufacturing practices, so that under normal and foreseeable conditions of use, they do not transfer their constituents to foodstuffs in quantities which could endanger human health, or bring about an unacceptable change in the composition of the foodstuffs, or bring about a deterioration in the organoleptic characteristics thereof. Inks, once printed on the non-food-contact si de of the packaging has to comply with the requirements of Article 3. Identification of potential migrants from printing ink used on paper and board food packaging material is a significant analytical challenge since the real formulations of printing inks are not known. Furthermore, more than 5000 substances can be used in packaging inks, according to the Swiss Federal Office of Public Health Ordinance (Annex 6 of RS 817.023.21). Consequently, large screening methods are needed to identify the substances used in these complex mixtures of printing inks. In this contribution, a method for the identification of potential migrants from printing inks used on paper and board food contact material is presented. The paper and board packaging was extracted with acetonitrile at 70°C for 24h followed by analysis using gas chromatography-mass spectrometry ($GC–MS^n$) in full scan and liquid chromatography coupled with Time-of-flight mass spectrometry (UPLC–ToF–MS) with both positive and negative electrospray ionization. Two approaches for the identification of unknown substances can be used. One approach is to focus on pre-selected target analytes, with known fragmentation patterns and retention times. In the presented work, a screening method for 139 analytes was developed using GC–MS and UPLC–ToF–MS. The substances in this targeted screening method are different additives, resins, solvents and photo-initiators, mentioned in the Swiss Ordinance. The other approach is untargeted screening in full scan mode using high resolution mass spec trometry. Afterwards, the unknown compounds are identified using existing GC-MS libraries, information of the elemental composition and collision induced dissociation (UPLC–ToF–MS). The developed method has already been used for the analysis of unprinted en printed paper and board and also the printing ink was analyzed. The results are discussed in this contribution.

Keywords: *Printing ink, paper and board, identification of unknowns, gas chromatography, ultra-high pressure liquid chromatography, high resolution mass spectrometry*

O-17
A NOVEL METHOD FOR EVALUATION OF MIGRATION OF FLUOROTELOMER ALCOHOLS FROM FOOD CONTACT MATERIALS TO FOOD

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Among many other applications of perfluoroalkyl and polyfluoroalkyl substances (PFASs), their unique properties make them convenient also for surface treatment of food contact materials. The commonly used surfactants for paper and board materials contain side groups based on fluorotelomer alcohols (FTOHs), which can potentially split off and migrate from the treated material to the food item. The probability of such reactions increases with growing temperature, and, thus, paper-based food contact materials with PFASs surface treatment may be significant source of FTOHs. The aim of this study was to develop a simple and effective method for determination of FTOHs in and board paper food contact materials and in some types of food, represented mainly by bakery products such as muffins. The head–space solid phase microextraction (HS–SPME) method was optimized for isolation of FTOHs from paper and board samples. The method was subsequently modified to obtain optimal parameters for determination of FTOHs in bakery products (muffins etc.).The identification of four analytes (2-perfluorobutylethanol –4:2 FTOH, 2-perfluorohexylethanol– 6:2 FTOH, 2-perfluorooktylethanol – 8:2 FTOH, 2-perfluorodecylethanol– 10:2 FTOH) was performed with gas chromatography (GC) coupled to a high resolutionmass spectrometric detector (MS) with a time-of-flight mass analyzer (TOF). The newly developed HS–SPME–GC–TOFMS method was used for determination of FTOHs in muffin cups and in muffin dough baked in (i) a silicone FTOHs–free form and (ii) paper cups containing FTOHs. The migration of FTOHs to muffins was proven as no FTOHs were detected in the muffins baked in silicone form whereas considerable amount of FTOHs was present in muffins baked in paper cups. Moreover, the amounts of 6:2, 8:2 and 10:2 FTOHs in muffin baked in “contaminated” cups remarkably exceeded the amounts of those FTOHs originally present in the muffin cup. This indicates the production of free FTOHs during baking procedure - most probably they are released from polyfluoroalkyl phosphate esters (PAPs).

Keywords: *FTOHs, HS–SPME, GC–MS*

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O-18
HUMAN DIETARY EXPOSURE TO BISPHENOL A: RESULTS OF THE SECOND FRENCH TOTAL DIET STUDY

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BPA-containing products are widely used in foodstuffs packaging as authorized within the European Union (UE n°10/2011). Therefore, foods and beverages are in contact with BPA which can migrate from food contact material to foodstuffs. In this context, an efficient analytical method using gas chromatography coupled to tandem mass spectrometry (GC–MS/MS), in the selected reaction monitoring (SRM) mode, was developed in order to quantify BPA in foodstuffs at very low levels (< 0.5 µg/kg). A drastic standard operating procedure, based on the combination of two successive solid phase extractions (SPE), was developed for various liquid and solid foodstuffs. The use of $^{13}C^{12}$-BPA as internal standard allowed accurate quantification of BPA by isotopic dilution. Several analytical pitfalls could be highlighted such as BPA extraction from freeze-drying sample and critical analytical parameters, i.e. background contamination and trueness of the measurement. Control ch arts based on both blank and certified materials have been implemented to ensure quality of the analysis. The developed method has been validated according to in-house validation requirements. Regarding linearity, correlation coefficients were above 0.99 within the range [0.1–100 µg/kg], the trueness of the method was assessed to 4.2%. Repeatability and within laboratory reproducibility were found in the range [7.5–19.0%] and [2.5–12.2%] for fortification levels of 0.5 µg/kg and 5.0 µg/kg, respectively, and in various matrices. The detection and quantification limits were evaluated respectively to 0.03 µg/kg and 0.10 µg/kg. A reporting limit was determined to 0.35 µg/kg, taking into account the mean background contamination. Finally the global uncertainty, expressed with a 95% confidence range, was determined as 20%. This protocol has been successfully implemented for the determination of more than one thousand foodstuff samples analyzed in the frame of a 2nd French To tal Diet Study. The majority of the samples (85%) presented contamination levels below 5 µg/kg. Remaining 15% of samples corresponded to canned products (vegetables, meat and fish based mixed dishes) and non-canned animal products. Mean contamination values amongst food groups ranged from 0.004 µg/kg for drinking water to 28.9 µg/kg for offal. Other groups with relatively high mean contamination levels are pulses, meat, fish and mixed dishes, at 14.7, 14.0, 11.9 and 8.6 µg/kg, respectively. Maximum values were found in offal (394.7 µg/kg) and meat (223.5 µg/kg). Based on these results, The French agency for food, environmental and occupational health safety (Anses) recommended taking measures to lower exposure of French pregnant women.

Keywords: *Bisphenol A, Total Diet Study, Human Exposure, GC–MS/MS,*

O-19
MIGRATION OF PHTHALATES FROM CARDBOARD BOXES AT THE EXAMPLE OF PACKED COFFEE FILTERS

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Bleached and unbleached filter papers are used daily for preparing coffee and tea beverages. As a matter of principle, coffee filter papers are manufactured from non-recycled paper. Consequently, they should be free of phthalates and other plasticizers. However, filter papers are usually offered in cardboard boxes which are produced of regenerated cellulosic fibers from which contaminants could potentially migrate to packed goods. For instance, phthalates and other softening agents are frequently employed in cardboard-prints. They are undesirable in food due to possible estrogen-like activities [1, 2]. By means of a fast GCMS method, we are able to quantify various phthalates in commercial coffee filter packaging boxes such as di-iso-butyl-phthalate, di-n-butyl-phthalate, di-ethylhexyl-phthalate and di-ethylhexyl-adipate etc. [3]. In this paper, we present results of systematic studies on the migration behavior of phthalates into filter papers. We demonstrate that the source of phthalate contamination is the recycled card box material itself rather than the printing color. The detected substances are capable to migrate from the packaging though the observed concentrations are approximately 100-fold lower in the filter papers. Highest amounts were measured in outside layers, which are in direct contact with the packaging. The time-dependency of migration rates has been revealed by means of long-term storage experiments using commercial coffee filter packs. Model brewing experiments with hot water were performed to assess a possible carry-over to coffee beverages. After a solid-phase enrichment step, the analytical method was sensitive to trace phthalates only in sub-ppb levels. In conclusion, the total intake of phthalates by consumption of filtered coffee is marginal compared to other sources.

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Keywords: *Phtalates, migration, coffee filter, packaging analysis*

PROCESSING CONTAMINANTS

(P-1 – P-19)

P-1
LEVEL OF DEGRADATION OF DEEP-FRYING OILS AND FATS FROM RESTAURANTS

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The aim of this study was to assess the deterioration of different types of vegetable oils and fats used in deep-frying various foods in 48 restaurants. Sunflower oil was the most widely used deep-frying medium (52.19%) in comparison with palm oil (16.65%), olive oil (12.50%) and oils of unknown origin (18.75%). Results for acid value (expressed as free fatty acids, FFA), total polar materials (TPM) and trans-fatty acids (TFA) content were reported and indicate oils and fats quality status under actual practices. TPM were examined by TESTO 270 (Testo, Germany), and FFA content was determined according to EN ISO 660/2009. TFA content was determined by Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR–ATR). For spectra acquisition, FTIR spectrometer Tensor 27 (Bruker Optics, Germany) was used. Spectral data were processed by Opus software (Bruker, Germany) for determination of TFA content, and UnscramblerX (CamoSoft, Norway) for chemometric analysis, which included multivariate curve resolution (MCR), principal components analysis (PCA), and principal component regression (PCR). Multiple linear regression (MLR) was used for the correlation between analyzed parameters.
Obtained results for acid value showed that only 11 samples (22.92%) did not exceed the permitted value (according to National Regulations), 10 samples of the sunflower oils and 1 sample of palm oil. The determination of the polar compounds and polymer triacylglycerols allow an objective evaluation of the thermal load conditions of deep-frying fats. According to the Working Group of Food Chemistry Experts – ALS (German Federal Health Gazette 2/91) the limit of rejection and replacement of cooking oil is 24% for TPM. TPM in 13 samples (27%) were over the limit of rejection and replacement, while 8 of them (16.67%) had TPM value higher than 30%. TPM in 14 (29.20%) were in the range 14-22%, which is considered as optimal range for frying.
The amounts of TFA in oil samples were from 0.07% to 40.88%. On average, the highest content of TFA was in sunflower oil, lower in olive oil and the lowest in the palm oil. PCR results showed a high correlation between the FTIR spectral data and the TPM content (R2=0.962). Based on MLR, it was shown that the increasing in TPM values during the heat treatment was a consequence of the hydrolysis of fats and of the increasing in the amount of FFA. Probability value for acid value ($p=0.001$) and for TFA ($p=0.014$) indicate the statistical significance of the influence ($p<0.05$) of these parameters on the measured values of TPM.
Obtained results showed that 27% of the examined samples of deep-fat frying oils and fats from restaurants in Belgrade exceeded the limited value (regarding TPM) and indicate the need for more frequent control and strict regulations by food authorities.

Keywords: *Deep frying, oils, fats, restaurants*

Acknowledgement: *This work was supported by grant from the Ministry of Education, Science and Technological Development of the Republic of Serbia (projects no. TR 031083)*

P-2
OCCURRENCE OF N-NITROSAMINES IN PROCESSED MEAT PRODUCTS ON THE DANISH MARKET AND DIETARY EXPOSURE ESTIMATES

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Nitrite and nitrate has for many decades been used for the preservation of meat. However, nitrite can react with secondary amines, present in the meat, to form N-Nitrosamines (NAs), many of which have been shown to be genotoxic [1]. The use of nitrite therefore ought to be limited as much as possible. To maintain a high level of protection for consumers, Denmark obtains national low limits for the use of nitrite in meat products.
Volatile NAs (VNA), are often found in relatively low amounts of ≤5 μg kg^{-1} [2], and have been the subject in the majority of the available literature. Knowledge of the occurrence of non-volatile NAs (NVNA) in food is limited. NVNA have been reported to occur in processed meat products at significantly higher levels than the VNA (up to 2000 μg kg^{-1}) [2].
The occurrence of eight VNA as well as six NVNA N-nitrosamines in processed meat products has been studied, primarily in products available on the Danish marked (n>55) but also in products purchased outside Denmark (n>15). A recently developed sensitive, selective and generic LC–(APCI/ESI) MS/MS method was used. Few samples were found to contain detectable levels of the VNA, whereas several of the NVNA occurred in the majority of the samples. Especially NTCA (N-nitrosothiazolidine-4-carboxylic acid) and NMTCA (N-nitroso-2-methylthiazolidine-4-carboxylic acid) were frequently found at levels ranging from <LOD to in the order of 2000 μg kg^{-1} and 30 μg kg^{-1}, respectively. The dietary intake of NAs (ng kg bw^{-1}) for children (4-5 years and 6-14 years) and adults (15-75 years) will be estimated from the obtained analytical data and consumption data from a Danish dietary study. The values will be presented on the poster. The highest intakes of NAs are expected for the 4 to 5 year old children, since this group has the highest intake of processed meat products in Denmark. For all three groups the intake of NTCA accounts for about the major part of the total intake.
Generally the intake of NAs is expected to be low; however the intake of NTCA and NMTCA are expected to be relatively high. Toxicological data on these two compounds are very limited and non-existing, respectively. The structures of NTCA and NMTCA are, very similar to N-nitrosoproline, which has been shown to be non-carcinogenic. However toxicological data on NTCA and NMTCA are needed before it can be rolled out that the intake of does not pose a health risk.

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[2] M.J. Hill, R.C. Massey, D.E.G. Shuker, S. Leach, A.R. Tricker, R. Preussmann, J.R. Rowland, 169pp. ISBN 0-89573-605-0 (1988).

Keywords: *N-Nitrosamines, nitrite, curing, processed meat, dietary intake, LC–MS/MS*

P-3
DETERMINATION OF PROCESS-INDUCED TOXICANTS AND ODORANTS IN FOOD BY MULTIDIMENSIONAL GC TECHNIQUES HYPHENATED WITH OLFACTOMETRY AND MASS SPECTROMETRY

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The assessment of the dual impact of heating treatments on food safety and aroma is a key issue. The objective of the present paper was the determination of process-induced polycyclic aromatic hydrocarbons (PAHs) and odor-active compounds with cooked meat as a food model. PAHs were analysed by accelerated solvent extraction - comprehensive bidimensional gas chromatography - time-of-flight mass spectrometry (ASE–GC×GC–TOF/MS). Odor-active compounds were determined by dynamic headspace - GC - eightbooth olfactometry (DH-GC-8O) and DH - multidimensional GC hyphenated with olfactometry and mass spectrometry (DH–GC–GC–-O/MS). For PAH determination, the GC×GC conditions consisted in a combination of a primary apolar BPX5 column and a secondary polar BPX50 column, and a modulation period of 5s. In terms of linearity, recovery rate and limit of quantification, the ASE–GC×GC–MS/TOF method was found consistent with the multi-residue determination of 17 PAHs in co oked meat. For aroma compounds, multi-booth olfactometry using eight sniffers revealed major meat odor-active compounds. A home-made heart-cut GC–GC–MS/O enabled to resolve the co-eluting odor zones with high odor-activity. Finally, these developments of multidimensional approaches were used to investigate and compare the balance between 17 PAHs and 68 odor-active compounds generated with different cooking techniques.

Keywords: *PAH, odor-active compounds; cooked food; GC×GC–TOF/MS; GC–8O/MS*

P-4
THE POTENTIAL TOXICITY OF RESIDUAL MICROBIOTA IN PRESERVED FOODS

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Foods after sterilization (pasteurization) can contain residual microbiota, which can include specific spoilage pathogens of canned foods; the bacterial pathogens, causing food poisonings; and the bacteria without affecting for the quality parameters, which approved regulations for production sterile canned foods. The later mentioned group include members of the microorganisms *Subtilis – Licheniformis* group: the aerobic spore-forming bacteria, which often found in the residual microbiota of canned products. Microorganisms of the *Subtilis – Licheniformis* group are the dominant raw material contaminants, and are found in the product before sterilized and detected in the residual microbiota of ready cans. The presence of aerobic bacillus does not always cause the significant changes of the organoleptic properties of canned products. The *Bacillus subtilis* - microorganisms that are used in some countries as probiotics and biopreserves. However, a review of the literature indicates the presence of *Bacillus subtilis* microbial strains with toxigenic properties even in the midst acceptable residual microbiota of canned foods, which can lead to decreased immunity and cause various diseases in humans. In experimental studies aerobic spore-forming bacteria were isolated from seven of the most popular canned. Among these canned were low acid canned vegetable - assortment and sauces. The samples of canned were sown, and the pure culture of microorganisms were been isolated from the sowing with the microbial growth. Studied the properties of these microorganisms can refer them to the *Subtilis – Licheniformis* group. The toxic effect of the isolated microorganisms *Subtilis - Licheniformis* group was studied with test-cultures *Stylonichia mytilus* and *Daphnia magna S.* The toxic effect of 4 isolates of aerobic spore-forming bacteria from the 18 isolates of aerobic spore-forming bacteria was detected. Dose-effect has been identified: the toxi city depend on the concentration of microbial cells *Subtilis – Licheniformis* group. For the same 18 isolated pure cultures of microorganisms *Subtilis - Licheniformis* were studied genotoxic and mutagenic activity of their metabolites with the of *Salmonella typhimureum* TA-98 and *Salmonella typhimurium* TA-100 strains (Ames test). The results, which were obtained using the spot-test with of Salmonella typhimureum TA-98 and *Salmonella typhimureum* TA-100 strains, were correlated with the results, which were obtained with the *Stylonichia mytilus*, *Daphnia magna S*. test cultures: for the same 4 bacterial isolates *Subtilis - Licheniformis* group with toxic effects were recorded expressed genotoxic and mutagenic activity of their metabolites. Thus, the analysis of residual microbiota composition in the most popular canned foods has revealed its potential danger. The representatives of microorganisms *Subtilis – Licheniformis* group were isolated, and their toxicity and DNA-damaging effect on the test-systems were found.

Keywords: *Potential toxicity, Subtilis – Licheniformis group*

P-5
RAPID SCREENING IMMUNOASSAY METHODS FOR ASSESSMENT OF ACRYLAMIDE IN FOOD

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Acrylamide is a controversial food contaminant with unclear effects on human health. Currently, the food industry and food safety regulatory bodies have been cooperating closely on approaches aimed at reducing acrylamide levels in processed foods to decrease human health risk. Therefore, rapid and inexpensive methods for acrylamide screening control in foods are required. The analytical performance and evaluation of a kit-based ELISA for the determination of acrylamide in fried potato, corn chip and cereal samples will be presented. The sample homogenate is subjected to clean-up using SPE, followed by analyte derivatization and ELISA detection. Accuracy, precision and linearity of the ELISA procedure has been validated using spiked samples. Analytical recovery ranged from 91.8 to 96.0% with coefficients of variation below 15%. Good linearity over a wide range of dilution and minimal assay drift was observed within a microtitre plate. IC50 value of the calibration curve was 110 ng/mL, with the limit of detection about 5 ng/mL and dynamic range from 10-1000 ng/mL. The high specificity of the ELISA was demonstrated by cross-reactivity study using eleven potential cross-reactants. A good correlation between the results obtained from ELISA and GC–MS and LC–MS/MS within the concentration range 120–1500 µg/Kg was found in the chip samples (r=0.992, n=120). Additionally, an evaluation of fluorescence polarization immunoassay based on monoclonal and polyclonal antibodies was conducted. The method is simpler and faster than ELISA not reguiring separation and washing steps. Another aim of the project was to develop an immunoassay – based lateral flow dipstick technology for rapid detection of acrylamide. Initial analytical data from the dipstick development will be shown. The data demonstrate that the evaluated and validated antibody-based methods have a potential utility in a quick, simple and reliable acrylamide screening analysis in the medium-and large-sized food industry companies, as well as for residues laboratories and the food industry dealing with improving the chemical safety of food available to the consumer.

Keywords: *Acrylamide, ELISA, polarization fluorescence, dipstick, rapid assay*

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P-6
POSSIBILITY OF PAHs ELIMINATION FROM SMOKED MEAT PRODUCTS BY ADSORPTION ONTO PLASTIC PACKAGES

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One of the important problems of food contamination is the presence of polycyclic aromatic hydrocarbons (PAHs) in food and their harmful effects on living organisms. Special attention is given to food products which are smoked or grilled because PAHs may be formed on their surface during the technological processing. This could affect safety and quality of food. Several papers have announced that the polymer packaging could play an important role in eliminating organic contaminants from food because of their high affinity to certain plastic materials. Lowering PAHs concentrations in polar and non-polar liquid media by adsorption on PET (polyethylene terephthalate) has already been unambiguously proven [1]. The aim of this study was testing the capability of commercially available plastic package material (PA/PE – polyamide/polyethylene) to eliminate PAHs from smoked meat products. On the basis of Commission Regulation (EU) No. 835/2011 PAH4 (the sum of the benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene) was chosen as a marker of occurrence of PAH in food. The changes of PAHs concentrations in smoked meat products packed in the plastic package were followed for 48 hours under controlled conditions in climate chamber and analyzed by high-performance liquid chromatography with fluorescence detection. Obtained results show that concentrations decreased as a reason of the interaction between PAHs in food and plastic packaging. It was due to the sorption of PAHs on packaging material, in which PAHs were found during the experiment and also at the end of the experiment.

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Keywords: *Polycyclic aromatic hydrocarbons; elimination, smoked meat products; plastic packages*

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P-7
ACCELERATED SOLVENT EXTRACTION WITH IN-CELL CLEAN UP – NEW EFFECTIVE SIMPLIFICATION OF BENZO[A]PYRENE ISOLATION FROM SMOKED SAUSAGES

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Accelerated solvent extraction (ASE) is a fully automated and reliable sample preparation technique that combines elevated temperature and pressures with liquid solvents to achieve fast and efficient removal of analytes from various matrices. ASE has many advantages over traditional extraction techniques, so it could be especially useful for routine analyses of polycyclic aromatic hydrocarbons (PAH) in smoked meat products. The aim of the work was to develop and verify a new simplified procedure for isolation of benzo[a]pyrene (BaP) from smoked sausages. The procedure applies extraction and clean-up operation in just one-step, thus avoiding losses of analyte and saving time, solvents and chemicals. To eliminate post-extraction clean-up step, silica gel as sorbent was applied directly to the ASE extraction cell to prevent the extraction of interfering compounds into BaP fraction. The procedure was studied at three content levels – 0.4, 5, and 10 $\mu g.kg^{-1}$. The spiked sample was placed into extraction cell on sorbent layer and extracted with n-hexane at 100°C and 10 MPa for 10 min. The flush volume was 60% and the purge time 120 s. One static cycle was accomplished three times. The extracts were evaporated to dryness, residuum was dissolved in methanol and analysed by HPLC. On the base of measured data, parameters of the procedure are as follows: R^2 was 0.99; LOD, resp. LOQ were 0.1 resp. 0.2 $\mu g.kg^{-1}$; Precision: values of Horrat coefficients HORRATR and HORRATr were less than 2 and recovery varied between 93–103 what is in the accordance with requirements set by Regulation (EC) No 2007/333. This new simplified procedure also shortens considerably time of determination and lowers handling with the sample.

Keywords: *Accelerated solvent extraction; polycyclic aromatic hydrocarbons; benzo[a]pyrene; smoked sausages; HPLC*

Acknowledgement: *This contribution is the result of the project supported by Science and Technology Assistance Agency of Slovak Republic under the contract No. APVV-0168-10.*

P-8
TRACE ANALYSIS OF NITROSAMINES IN BEER USING GC–MS/MS

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Nitrosamines are in common highly toxic compounds with high cancerogenity for humans and animals as well. A large variety of nitrosamine compounds are known and described with different alkyl moieties. The simplest N-nitrosodialkylamine with two methyl groups is the N-nitrosodimethylamine (NDMA), described as a very potent carcinogen occurring in several food preparations but also known as being generated during the brewing process. The source of nitrosamines in the brewing process is described by the reaction of nitrogen oxides with alkaloids from the drying process of the germinated malt in beer production. In addition to the regular control of food products for daily consumption, malt in beer production is also monitored routinely for low levels of nitrosamines. As nitrosamine levels in malt and beer have been significantly reduced in the modern brewing process, high analytical performance and selectivity for matrix loaded samples is required. This application describes a new GC-MS/MS method for routine detection and quantitation of food borne nitrosamine compounds. The sample preparation is adapted and slightly modified from AOAC Official Method (2000), 982.11. An SPE column extraction method using a celite column and elution with DCM to isolate the nitrosamines from the beer samples was developed. For GC-MS/MS detection of the nitrosamine compounds the EI ionization was used in contrast to earlier publications and the EPA method 521, which were applying liquid CI conditions on an ion trap instrument. The applied MRM method using a triple quadrupole GC–MS/MS instrument provided very high matrix selectivity even for the small nitrosamine target molecules and compound specific low mass fragment ions. All nitrosamine compounds in the current study could be determined with LOD below 1 ppb, using 1 ppb as the lowest concentration for the quantitative calibration. The GC–MS/MS system applied showed a wide linearity in the range of 1–500 ppb with very good precision. All calibration curves have been shown to be strictly linear with R^2 better than 0.99.

Keywords: *Nitrosamines, food, EI ionization, MRM method, quantitation, LOQ, linearity*

P-9
DETERMINATION OF 3-MCPD DIESTERS AND MONOESTERS IN VEGETABLE OILS AND FATS

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The concern raised by the high levels of fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD esters) in refined vegetable oils and fats prompted global investigations on the methods used to analyze these contaminants. Several indirect methods, performed under acid or alkaline conditions, have been proposed. In general, these methods present high sensitivity and do not require a large series of standard compounds. However, since they are used to determine the total amount of 3-MCPD esters, it is not possible to identify the different species of the compounds, i.e., diesters and monoesters. The quantification of 3-MCPD diesters and monoesters is important in order to assess the contribution of foods to the bioavailability of 3-MCPD, which could clearly provide more information of use in toxicological studies and risk assessment. Therefore, the aim of this work is to propose the application of solid phase extraction (SPE) on silica cartridges to separate 3-MCPD diesters and monoesters before the transesterification step employed in indirect analytical procedures, and determine the levels of the compounds in commercial samples of oils and fats from the Brazilian market. The sample preparation included the dilution of the sample in hexane, separation of 3-MCPD diesters and monoesters on HF Mega BE-SI 1gm/6mL silica cartridges (Agilent Technologies), transesterification employing a mixture of sulfuric acid and methanol, neutralization by adding sodium hydrogencarbonate solution, salting-out of lipophilic compounds using ammonium sulfate solution and hexane, derivatization of the released 3-MCPD with phenylboronic acid and analysis by gas chromatography coupled to mass spectrometry. The analytical method showed good linearity in the range of 0-4 mg/kg (r2 = 0.999). No matrix effect was observed. Limits of detection (LOD) and quantification (LOQ) were calculated as 0.1 and 0.2 mg/kg, respectively. Mean recoveries varied from 74 to 98% and coefficients of variation ranged from 6.9 to 11.5% for repeatability and from 6.8 to 16.2% for within-laboratory reproducibility. The method was applied to determine the content of 3-MCPD diesters and monoesters in 61 samples of vegetable oils and fats marketed in Brazil. The levels of diesters varied from not detected (nd) to 3.52 mg/kg and represented from 35 to 96% of the total 3-MCPD esters (considering 45 samples with concentrations above LOQ). The levels of monoesters ranged from nd to 2.63 mg/kg and represented from 7 to 65% of the total concentration of 3-MCPD esters (considering 23 samples with concentrations above LOQ). In conclusion, the validation results indicated the reliability of the proposed method for the determination of 3-MCPD diesters and monoesters in vegetable oils and fats. The analysis of real samples showed that the 3-MCPD esters were predominantly present as diesters.

Keywords: *3-MCPD esters, solid phase extraction (SPE), refined oils*

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P-10
2- AND 3-MONOCHLOROPROPANEDIOL AND GLYCIDOL FATTY ACID ESTERS IN FOODS

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Recent studies have identified the presence of 2- and 3-monochloropropanediol and glycidol bound in the form of fatty acid esters - (2- and 3-MCPDEs and GEs) - in many refined fats and oils and also food products manufactured with fats and oils, such as cookies. 2- and 3-MCPD and glycidol esters may be formed during processing/refining of commercials oils. Some fatty acid esters of 2- and 3-MCPD and glycidol can be hydrolysed to their respective parent compounds, glycidol and 2- and 3-MCPDs. Glycidol is a multisite carcinogen in rodents while 3-MCPD is classified as a non-genotoxic carcinogen. The toxicological significance of 2-MCPD is unknown due to a lack of data but its presence in food is of interest based on structural similarities with 3-MCPD. Until now there were no data on levels of those contaminants in food products on the Canadian market. A new method based on LC–MS/MS was developed. The new method incorporates isotope dilution for quantifying the five analytes: glycidol esters of palmitic, stearic, oleic, linoleic and linolenic acid. The method was later extended to include analysis of glycidol esters of lauric and myristic acid. The new method for analysis of 2- and 3-MCPD via derivatization with cyclohexanone/solid phase catalyst was also developed. We are presenting data on the occurrence of 2- and 3-MCPDEs and GEs in more than 50 products of edible fats, oils and related products containing fats/oils such as cookies and cooking sprays on the Canadian market. Most of those products were purchased from retail stores in Ottawa between 2011 and 2013 in duplicate thus allowing for evaluation of temporal trends. Oils labelled as virgin/unprocessed did not contain detectable levels of GEs or MCPDEs or contained them in trace amounts. GEs and MCPDEs content was highly variable in processed oils/fats, reaching 10.6 and 17.1 mg/kg (expressed as sum of target GEs and MCPDs equivalents, respectively). Health Canada's Food Directorate will use the data to update its exposure estimations and risk assessment for 2- and 3-MCPD and glycidol esters in food.

Keywords: *2-MCPD, 3-MCPD, glycidol, esters, foods*

P-11
QUANTIFICATION OF 15+1 EU PRIORITY PAH IN REPRESENTATIVE GERMAN SMOKED MEAT PRODUCTS USING A FAST GC–HRMS METHOD.

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For the analysis of the 15 polycyclic aromatic hydrocarbons (PAH) classified as priority from the Scientific Committee on Food (SCF) and benzo[c]fluorene assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA) in food, a sensitive analytical method is necessary. Therefore at the Max Rubner-Institut (MRI) in Kulmbach a Fast-GC–HRMS method with a runtime of only 25 minutes using a TR-50ms column (10 m × 0.1 mm × 0.1 µm) was developed. 113 representative samples of commercial smoked German meat products were tested. The median of benzo[a]pyrene content was 0.03 µg/kg and therefore greater than a factor of 100 below the maximum level of 5 µg/kg. The required analytical parameters limit of detection (LOD) and limit of quantification (LOQ) for this type of low level samples can be achieved with a high resolution sector field instrument for all individual PAH. The determined LODs and LOQs were in the range of 0.003–0.01µ/kg and 0.009–0.03 µg/kg, respectively. The correlation coefficient (R) between benzo[a]pyrene and the sum of the 15+1 EU priority PAH was 0.90. The analysis of meat reference material with spiked concentrations in the range of 4.1 to 9.9 ug/kg resulted in recoveries from 68 to 113%.

Keywords: *GC/HRMS, priority PAHs, smoked meat products, fast GC*

P-12
THE ROLE OF HYGIENE OF ENVIRONMENTAL SURFACES IN PRODUCTION PLANTS IN THE PREVENTION OF MICROBIOLOGICAL CONTAMINATION OF FRESH-CUT VEGETABLES

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There are several possible sources of microbiological contamination of fresh vegetables, from cultivation to storage facilities, food processing factories, private homes and professional kitchens. Hygiene in production plants of fresh-cut vegetables is an essential factor affecting the microbiological quality of these vegetables. Biofilms on environmental surfaces of the production plants may enhance survival of pathogens. In our hygiene monitoring studies several potential points for microbiological and organic contamination of the vegetable products were identified. High levels of total aerobic microbes, yeasts and enterobacteria were detected on vegetable processing machines, and high values of ATP were detected e.g. on some packages and cutters. Microbial contamination of the fresh-cut vegetables can partly be prevented, and general environmental hygiene be promoted in the processing plants by appropriate planning and design of the factory and equipment, by managing moisture, temperature and particles in air, and by cleaning the surfaces efficiently. In addition self-monitoring systems are essential for successful management of process hygiene. Since fresh-cut vegetable production is a labour-intensive branch, human factors in promoting product and process hygiene must always be taken into account and personnel must be trained regularly. Our studies showed that enhancements carried out in the production plants can result in improved hygiene in environmental surfaces in these plants.

Keywords: *Vegetable, processing, hygiene, environment, surface*

P-13
REDUCTION OF CARCINOGENIC 4(5)-METHYLIMIDAZOLE IN GLUCOSE-AMMONIUM HYDROXIDE MODEL SYSTEM: INFLUENCE OF THE FOOD ADDITIVES

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4-Methylimidazole has received much attention from many researchers and regulatory agencies because of its carcinogenicity. In present study, the effect of additives on formation of carcinogenic 4(5)-Methylimidazole (4-MI) in glucose/ammonium hydroxide model system was investigated. Aqueous model solutions were treated by heat at 100°C for 2 h and determination of 4-MI formed were performed using gas chromatography-mass spectrometry (GC–MS). The formation levels of 4-MI ranged from 110.9 to 656.7 ppm. When the D-glucose and ammonium hydroxide was heated, amount of 4-MI formed was 556±1.30 mg/kg and increased to 583±2.61 mg/kg (5% increase) from addition of sodium sulfite 0.1M. In the other systems added food additives which are Fe^{2+}, Zn^{2+}, Mg^{2+}, cystein, tryptophan at level of 0.1M, however, the amount of 4-MI were reduced as compared to glucose/ammonium hydroxide system. The formation level and reduction rate of 4-MI by addition of additives were 11 0±0.73 mg/kg (80% decrease) in 0.1M Fe^{2+}, 483±2.07 mg/kg (13% decrease) in 0.1M Mg^{2+}, 460±2.06 mg/kg (17% decrease) in 0.1M Zn^{2+}, 409 ± 4.43 mg/kg (26% decrease) in 0.1M tryptophan, and 397±1.71 mg/kg (29% decrease) in 0.1M cysteine. The greatest reduction was occurred in the system with Fe^{2+} by 80% and the degree of contribution to reduction of 4MI from food additives has the following order: Fe^{2+} > cysteine > tryptophan > Zn^{2+} > Mg^{2+}. As the amount of additives decreased below 0.1M the amount of 4-MI increased, suggesting that the presence of additives affect the formation of 4-MI. Also, color and flavor value were evaluated in terms of responsible factors of Maillard reaction products. Colors among the system solutions measured by a colorimeter were not significantly different. Whereas additives have effect on the level of pyrazine formed in Maillard reaction. 4-MI content, in the model system solutions, have correlation to formation of 2-Ethyl-6-methylpyrazi ne (R^2 = 0.7215).

Keywords: *4(5)-Methylimidazole, Maillard reaction, Food additives, Model system*

P-14
DETERMINATION OF ACRYLAMIDE IN POTATO CHIPS SAMPLES USING DIFFERENT ANALYTICAL TECHNIQUES

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Acrylamide was formed in some foods cooked at high temperature (120–170°C) by the reaction of the amino acid asparagines with a reducing sugar such as glucose. Acrylamide is genotoxic and carcinogenic in studies in animals. It causes increased tumour incidence at a variety of sites. The calibration graphs are linear over the ranges 1.0–8.0 and 2.0–13 µg/ml of acrylamide, with detection limits of 0.6 and 1.0 µg/ml, respectively. The methods are applied to the routine analysis of acrylamide in potato chips samples. It confirms that the analytical procedure employed for the analysis is suitable and reliable for its intended use. Acrylamide was also determined in the presence of glucose and asparagines. Reduction of some cationic interference was carried out in the batch and flow injection analysis using cationic exchanger of hydrogen form with 2.5 –mm internal diameter, 15-cm length of packing and flow rate 0.5 ml/min. The proposed first and second derivative methods for de termination of acrylamide, are simple, rapid (as it only requires measurements of nD values at a single wavelength). They were used for identification of the acrylamide depending upon characteristic peaks at certain wavelengths or ranges. The first and second derivative spectra of the mentioned compound have been used for determination of the compounds at different ranges of concentration depending upon the measurements of the heights of the peak to the baseline at certain wavelengths. HPLC was used as a standard method for qualification and quatitation of acryl amide in different potato chips samples, and the results obtained were agreed with those of the proposed methods.

Keywords: *Determination, acrylamide, potato chips, analytical techniques*

P-15
INVESTIGATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS AND STRATEGIES OF THEIR DECREASE IN SMOKED MEAT PRODUCTS

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Smoke fume is generated during wood pyrolysis. The basis for pyrolysis is the free radical reactions of thermodestruction of hemicellulose, cellulose and lignin, taking place at a temperature higher than 200°C. The constitutive parts of wood, are turned therewith to phenols, alcohols and carbonyl compounds. However, smoking of food products is accompanied by the development of the toxicologically harmful smoke components, namely, polycyclic aromatic hydrocarbons (PAHs). The high carcinogenic activity of minor PAHs, their chemical stability and the synergetic effect of the several PAHs exposure confirm the necessity of their tough control in meat products. The investigations of the treatment of the fatback samples with phenols isolated from liquid smoking preparations showed that as phenolic substances dissolve into a fat fraction of fatback consisted mainly of saturated triglycerides, on the average, about 75% of the phenolic substances of the preparation were not identified later on. On the basis of the analysis of the PAHs quantitative content, however, it was shown that in raw smoked sausages with backfat in their composition, the PAHs residual quantity is up to 30% higher than in the products of this type of smoking, which composition did not include backfat. The increase in temperature led to the increased CO and CO_2 content and the reduction of the oxygen concentration. The PAHs quantitative content grew in direct proportion as the smoke generation temperature and the CO and CO_2 concentrations were increasing and in inverse proportion to the oxygen concentration. Analysis of the PAHs quantitative content in meat produce in a framework of the certification showed that 8 PAHs namely benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene, indene[1,2,3-cd]pyrene were most frequently detected. On the basis of the research results, it can be concluded that it is possible to achieve the PAHs content reduction in several ways. One of them is the reduction of fat content in the formulation of smoked products. With that, the PAHs content reduction is not associated with the reduction of phenolic substances, which are responsible for taste and aroma of smoked products. The main parameter influencing the PAHs generation is the temperature of the smoke generation. The advantages of use of protein and, especially, fibrouse casings compared to natural casings are evident from the obtained data. Protein and fibrouse casings are denser, their protein-cellulose base is not highly fat swelling, but the PAHs penetrability through such barriers is in many respects hampered. The performed preliminary investigations showed that a number of ingredients assist the production of smoked meat products with reduced PAHs content. These ingredients include many kinds of spices, ascorbic acid and a variety of natural stabilizers.

Keywords: *PAH, phenols, smoked meat products*

P-16
POTENTIAL OF FRONT-FACE FLUORESCENCE BASED FLUORALYS® ANALYZER TO MONITOR ACRYLAMIDE AND COLOR OF POTATO CRISPS

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In 2002, acrylamide has been identified as a major contaminant in some severely heat-treated food products. This is particularly the case for potato products, and especially potato crisps. Currently, the most common method to measure acrylamide content in potato crisps is liquid or gas chromatography coupled to mass spectrometry. But such techniques are expensive and time-consuming. Rapid and simple methods are needed to be implemented near the manufacturing plants to comply with European Commission guidevalues. In addition, rapid techniques can help developing acrylamide mitigating strategies that preserve the sensorial profile of the product. We present the Fluoralys® analyzer, based on a new front-face fluorescence technology, and demonstrate its potential to simultaneously predict color and acrylamide in potato crisps. Starting from 3 different varieties of potatoes with various levels of asparagine and reducing sugars, we managed to establish satisfyi ng calibration models over acrylamide content, thanks to multilinear regression between PARAFAC decomposition scores and the chromatographically measured acrylamide or colorimetric data. 100 samples were analyzed over 9 months for water content, color and acrylamide using both ELISA and LC-MSMS. The three varieties were stored for different times and were from different geographical origin. The frying process was carried out on 3 different frying tunnels, so that the total variability was high. A first PCA allowed identifying the structure of the variables and building some groups essentially driven by the potato variety. One model per variety was firstly built with satisfactory prediction quality. We discuss how these models can be merged to develop a general model and the level of robustness of the prediction by analyzing the prediction error observed on new samples. The color was similarly predicted, allowing comparison of acrylamide levels for a given target color. In conclusion, front face fluorescence, and in particular Fluoralys® analyzer, seems a powerful tool to predict in real time color and acrylamide content of potato crisps for routine quality control at the production line.

Keywords: *Acrylamide, fluorescence, potato crisps, color, prediction model*

P-17
ROLE OF SUGARS ON THE FORMATION OF ACRYLAMIDE AND 5-HYDROXYMETHYLFURFURAL IN BISCUITS

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Sugar is one of the main ingredients of sweet bakery products. Under heat treatment, reducing sugars such as glucose and fructose react with free amino acids in the Maillard reaction to generate the desired product taste and colour. However, unwanted compounds such as acrylamide and 5-(hydroxy)-2-methylfurfural (HMF) can be also formed in the baking process and be present in the final products. The aim of this study was to investigate the role of sugars in the formation of acrylamide and HMF during baking biscuits. Three different biscuit recipes were used, varying in sugar types, particularly sucrose, glucose and fructose. All other ingredients of the three recipes were the same. The biscuits were baked at 200°C for 16 minutes. Every two minutes, biscuits were collected from the oven and chemically analysed for sucrose, glucose, fructose, asparagine, acrylamide and HMF. The results showed that during the baking process, in all three biscuit recipes, concentrations of sugars and asparagine decreased, whereas concentrations of acrylamide and HMF increased. At the end of the baking process, biscuits prepared with sucrose had much lower levels of acrylamide and HMF than biscuits containing reducing sugars. Biscuits prepared with only fructose (17.5 g) showed the highest concentration of acrylamide of all three biscuit recipes. The biscuits prepared with a 1:1 mixture of fructose (17.5 g) and glucose (17.5 g) showed the highest HMF concentration of all three biscuit recipes. The concentration of HMF in these biscuits was ten times as high as compared to biscuits prepared with only fructose. The very low concentrations of acrylamide and HMF in the biscuits prepared with sucrose can be explained by the lag phase before sucrose started to hydrolyse into glucose and fructose. We conclude that the ratio of fructose to glucose as ingredients of biscuit s can influence the concentration of acrylamide and HMF in the final products. Also, the acrylamide and HMF formation in bakery products can be minimized if fructose and glucose in the dough are replaced by sucrose.

Keywords: *Acrylamide, sucrose, frucotse, glucose, biscuits*

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P-18
MONITORING THE QUALITY OF CZECH COMMERCIAL POTATO CRISPS APPLYING DART–HRMS AND U-HPLC–MS

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During thermal processing of food, such as deep-fat frying, the foodstuff and frying oil undergo many chemical reactions supporting the formation of desirable compounds effecting the flavor, texture and color of the product. Nevertheless, undesirable compounds like e.g. oxidized triacylglycerols (TAGs) are also formed in the frying oil and may pose health hazard to consumers. Besides that, the oil may contain significant amounts of 3-monochloropropane-1,2-diol esters (3-MCPD), which are according to recent studies already particularly formed in vegetable oils during refining in the deodorisation process. There is a suspicion that 3-MCPD esters may undergo a lipase-catalyzed hydrolysis in the human gastrointestinal tract and so free 3-MCPD (a possible carcinogen, group 2B) can be released. In this study we demonstrate two novel instrumental approaches to monitor the quality of the oil present in commercially available potato crisps. Potato crisps were selected for analysis because of their very high consumption and presence of processing contaminants. Direct Analysis in Real Time (DART) hyphenated to the (ultra)high resolution mass spectrometry (HRMS) was used to monitor oxidized non-polar TAGs and their oxidation products in the oil extracted from the potato crisps with toluene 1:10 (*w/v*). To enhance the ionization of TAGs, a 2 mL vial containing an aqueous solution of ammonia (25%, w/w) was placed at a distance of 90 mm from the DART gun exit. To visualize the differences of oxidized TAGs and their b-scission products in each sample, multivariate statistical analysis (principal component analysis, PCA) was used. Ultra-high pressure liquid chromatography (U-HPLC) coupled to a high-resolution spectrometry (HRMS) employing an Orbitrap mass analyser was applied to determine individual native 3-MCPD diesters. Before the instrumental approach, a clean-up step on silica gel mini-column chromatography was used to remove the interfering TAGs that induce strong matrix effects. The quantitative analysis was performed with the use of deuterium-labeled internal standards.

Keywords: *Oil, Oxidation, 3-MCPD, Mass spectrometry, Direct Analysis in Real Time*

Acknowledgement: *Financial Support from Specific University Research (MSMT No. 20/2013).*

P-19
ACRYLAMIDE FORMATION IN TRADITIONAL CZECH LEAVENED WHEAT-RYE BREADS AND WHEAT ROLLS

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The main aim of our study was to collect the data on the acrylamide content in typical Czech leavened wheat-rye breads and wheat rolls, and to demonstrate the influence of various technological factors on its contents. We analysed several sets of industrially and laboratory baked breads to better understand the acrylamide formation. Following factors were tested: *(i)* the content of rye in respective bread formulae and *(ii)* the character of leavening procedure. We were, as well, monitoring for ten days the variability of acrylamide levels in bread baked by one bakery and, afterwards, we analysed the same type of bread baked in 12 bakeries. In the case of rolls, the influence of yeasts and vital gluten content in the dough were subjects of an assessment. The levels of acrylamide in a common type of the Czech bread baked in various bakeries were relatively low, ranging from 7 to 22 $\mu g.kg^{-1}$. The content of acrylamide was rather higher in breads prepared using a commercial starter (Lactobacilli) and yeasts than in bread prepared using natural rye sourdough only. A varying content of rye flour did not seem to have any impact on acrylamide levels. The ´size dilution effect´ was observed when comparing the same type of bread with different weight. Since acrylamide is exclusively located in the bread crust, smaller loaves contained higher acrylamide content per kg. The contents of acrylamide in standard wheat bakery goods were very low, bellow 10 $\mu g.kg^{-1}$. No influence of varying content of added yeast or vital gluten was observed.

Keywords: *Acrylamide, bread, flour, sourdough, yeasts*

Acknowledgement: *This study was carried out with the support from the following projects financed by the Ministry of Education, Youth and Sports of the Czech Republic: (i) the NPV II. project 2B06168; (ii) project MEB 080882 and (iii) Specific University Research (MSMT No. 20/2013).*

RESIDUES

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PESTICIDES

(R-1 – R-69)

R-1
DISTRIBUTION OF PESTICIDE RESIDUE WITHIN A PAPAYA FRUIT AND ASSESSMENT OF SAMPLE PREPARATION ERROR

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A sample received at laboratory not including package is known as laboratory sample. Conversion of laboratory sample in to analytical sample may need sample preparation which includes removal of parts such as soil, stones, bones, withered leaves, etc. Since Maximum Residue Levels (MRL) are set for residues present in the whole commodity including inedible peel and the outer inedible portions, sample preparation step should not remove these inedible portions. On the other hand, the level of residues in the edible portion of a food commodity is used for estimating dietary intake values. Sample preparation may be subject to systematic and random errors that cannot be estimated. Therefore, each analyst should exactly follow the proper procedure. In the scope of this study, the possible error deriving from sample preparation was demonstrated with the analysis of residues in peel and pulp of papaya treated postharvest with benomyl, a systemic fungicide, a few day s before the laboratory analysis. In the present study, each papaya unit were cut in to 4 concentric sections in longitudinal directions. Two opposite sections were taken for processing and the other two were discarded. Then, peel, pulp and the seeds of the selected sections were separated for further processing. In order to determine distribution of benomyl (a systemic pesticide) in edible and inedible portion of 11 papaya samples, peel and pulp of were homogenized separately and analyzed with HPLC equipped with UV detector. The reliability of the results in every analytical batch was monitored and confirmed with recovery tests and analysis of replicate test portions. Benomyl residue, measured as carbendazim, in corresponding sections of peel and pulp of papaya samples ranged from 0.178 mg/kg to 1.325 mg/kg and 0.025 to 0.087 mg/kg, respectively. Benomyl concentration decreased in a range between 41% and 83% by peeling of papaya. As the penetration of the pesticide from the surface to the peel and the pulp was limited due to short time between treatment and analysis, the distribution of residues was uneven. Consequently, the proportion of peel and pulp after their separation significantly affected the residues measured in peel and pulp. Thus, the selected test system could be well used to demonstrate the possible variability of measured residues depending on the uniformity of sample preparation. MRL for benomyl is 0.2 mg/kg which is expressed as carbendazim. For example, one of the slices taken from papaya sample has residue value as 0.208 mg/kg slightly above the MRL, whereas residues in its pulps were not exceeding MRL. Furthermore, ca rbendazim residues in all papaya peels were above the MRL values in the range of 0.728 to 1.325 mg/kg. Therefore, pesticide residue analysis should be carried out accurately in peel, pulp, or in whole fruit taking into consideration purpose of the analysis and the proper sample preparation protocol.

Keywords: *Pesticide residues, sample preperation, distribution, benomyl, papaya*

Acknowledgement: *The present study was performed in FAO/IAEA Training and Reference Center for Food and Pesticide Control, Austria. The support of FAO/IAEA in carrying out the study and the member of IAEA Agrochemical Units in Vienna are greatly appreciated.*

R-2
EVALUATION OF ATMOSPHERIC PRESSURE IONIZATION SOURCES (ESI, APCI AND APPI) IN LC–MS/MS FOR SELECTED PESTICIDE RESIDUE ANALYSIS IN CUCUMBER

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Food safety and effective functioning of the internal markets are key concerns of citizens and authorities. To control the quality of the food we are consuming the EU applies an integrated approach that includes legislation, standards, guidance documents or monitoring programs. In the field of pesticide residues, EU legislation establishes Maximum Residue Limits (MRL) allowed in different commodities, which are regularly monitored by e.g. multiannual control program in the Union (2). Adequate analytical tools are essential to measure correctly the levels of pesticides, and guidance is available for method development (3) and validation (4). Ideal tools for the assessment of the level of accuracy of the analytical measurements are certified reference materials (CRMs). These well characterised matrix materials may be certified by means of highly accurate analytical methods. As part of a project towards the production of a CRM, the IRMM is developing analytical methods for the quantification of selected pesticides in cucumber, focusing on the achievement of the highest accurate measurements and minimum associated uncertainty. Steps such as extraction and clean-up are usually contributing significantly to the total uncertainty of the methods (5). Thus, the simplification of the sample treatment may be considered as strategic approach to achieve lower uncertainty values. Nowadays, the sample treatment has been reduced drastically for food matrices in LC–MS/MS analysis (6,7). Since the last 20 years, electrospray ionization (ESI) was the most commonly used ionization source to couple to LC–MS/MS systems in polar smal l molecules analysis (8). Nevertheless, some families of pesticides that are still applied in the field are less polar, and then poorly ionisable in ESI source. Therefore, the use of other atmospheric pressure ionization sources than ESI could alternatively be considered for a wide range of different chemical group of pesticides in a single analysis, such as atmospheric pressure chemical ionization (APCI) and specially atmospheric pressure photo ionization (APPI). The aim of this study is to evaluate the behaviour of a representative group of pesticides in a vegetable matrix, such as cucumber, when three different ionization sources are used (ESI, APCI and APPI) in LC–MS/MS, in order to obtain the most trustworthiness analytical method for calculating the relative uncertainty of a new CRM. The study can be divided in the next objectives: *(i)* to develop a new analytical method based on LC-ESI-MS/MS using a simple sample treatment; *(ii)* to investigate how APCI and APPI sources can expand the scope of the method to less polar pesticides; *(iii)* to study the sensitivity, reproducibility and matrix effect of the method depending on the ionization source; and, *(iv)* to calculate and compare the relative uncertainty values of the analytical method depending on the sample processing and determination approach.

Keywords: *Pesticides, liquid chromatography-mass spectrometry, chemical ionisation, photoionisation, certified reference material*
Acknowledgement: *The IRMM-SID processing team is acknowledged for the preparation of the cucumber material employed in the study and EURL FVs for their contribution in the selection of target pesticides.*

R-3
APPLICATION OF GAS CHROMATOGRAPHY TIME-OF-FLIGHT HRMS MASS SPECTROMETRY TO FOOD ANALYSIS

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During the last years the introduction of liquid chromatography high resolution mass spectrometry (LC–HRMS) has become popular in pesticide residues laboratories. The advantages in getting exact mass of the analytes have been evaluated giving an important and new solution to common problems of these analyses. But, till now practically all applications have been focused on LC [1,2]. Recently new GC–HRMS are intending to cover a similar position for typical GC pesticide residues. Some questions remain to clarify if the established workflows in LC can be similar for GC–HRMS considering of the differences between ionization systems as ESI or EI. This work reports the development and evaluation of a rapid automated screening method for determining pesticide residues in food using gas chromatography time-of-flight mass spectrometry (GC−TOF–HRMS) based on the use of an accurate-mass database. The database (including 80 GC amenable pesticides) created include, ions obtained under electron impact ionization at 70 eV and retention times of each pesticide under retention time looking of pesticides at constant flow. This customized database was associated to commercially available software which extracted all the potential compounds of interest from the GC−TOF HRMS MS raw data of each sample and matched them against the database to search for targeted compounds in the sample. Manual and automatic identification with the developed workflow has been tested in tomato, orange, potato and onion extracts spiked at 10 and 100 ppb levels, all pesticides present in the sample were identified correctly by using as identification criteria; (i) retention time window of 0.5 min, and (ii) a mass error tolerance of 10 ppm for at least two fragments selected for each pesticide. A new method has been validated in the four matrices commented. The validation parameters were adequate for residue analysis. This work pointed out the potential of this new analytical tool in food control laboratories.

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[2] Malato, O., Lozano, A., Mezcua, M., Agüera, A., Fernandez-Alba, A.R. Journal of Chromatography A 1218 (42), pp. 7615–7626 (2011).

Keywords: *Pesticides, GC–TOF, HRMS*

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R-4
DETERMINATION OF SULFURYL(DI)FLUORIDE THROUGH SULFURYLCHLORID FLUORIDE IN FOOD SAMPLES USING HS-SPME-GC/MS

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Sulfuryl fluoride (SO_2F_2) is registered as a fumigant for the control of insect pests in closed structures (grain processing facilities, domestic dwellings, garages, barns, storage buildings, etc.) and their contents (cereal grains, dried fruits, tree nuts, etc.) In July 2005, EPA approved sulfuryl fluoride for the direct treatment of additional harvested and processed food commodities such as coffee and cocoa beans, and for the fumigation of food handling and processing facilities. It' use increased rapidly, replacing methyl bromide, now being phased out because of the harm it does to the ozone layer. It is also an alternative to phosphine. This substance is very difficult and dangerous to handle. Our proposal: analysis of sulfurylchloride fluoride (SO_2ClF), which is less dangerous than sulfurylfluoride and easier to handle. And we can easily synthesize it fresh in the lab using fluorination of sulfuryl(di)chloride ($S0_2Cl_2$) with NaF in presence of acetonotrile. The fragment SO_2F is the same for both compounds. By scanning this segment we can determine if sulfurylfluorid is present. We can use sulfurylchloride fluoride for quantification and SPME control. The calibration of the sulfurylchlorid fluoride takes place through sulfuryldichloride which is liquid and available as a certificated standard. Reproducibility of the sulfurylchloride fluoride synthesis was tested via its tenfold synthesis on three days. The standard deviation was 9%. It means that the synthesis is stable and reproducible. The LOQ of our method is 0.005 mg/kg for each commodity Linearity range: 0.005 mg/kg to 0.5 mg/kg Recovery rate range: 75–110% and the measurement uncertainly: +/- 50%

Keywords: *Fumigants, food, residues, sulfurylfluoride*

R-5
HIGHLY SENSITIVE DETERMINATION OF PHOSPHINE IN FOOD

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To protect food in silos or sea containers from attack by feeding pests such as moths, beetles and mites, fumigants like phosphine and sulfuryl fluoride are used, among others. For conventional foods, limits between 0.01 and 0.1 mg/kg are applied in the EU for the sum of phosphine and phosphide. In organic cultivation the use of phosphine in empty containers and silos is allowed, but not for the food itself. To recognize and avoid cross contamination, particularly through dust, it is necessary to use a sensitive detection method for phosphide and phosphine. [1] The acceptance of organic food containing over 0.001 mg kg is currently being questioned in Switzerland. Outside of Switzerland the BNN orientation value is > 0.01 mg/kg. Phosphine is in the acid desorbed from the sample matrix. Using the method of Scudamore and Goodship existing phosphides are converted to phosphine. [2] An aliquot of the gas phase is removed for assay and analyzed by HS–GC / FPD. The calibration is carried out using a recognized external standard like Zn_3P_2. This standard is generated directly in a headspace vial and the different dilution steps of the calibration were automatically done by the Gerstel injector of the measurement system.
The LOQ of our method is 0.0003 mg/kg for each commodity
Linearity range: 0.0003 mg/kg to 0.5 mg/kg
Measurement uncertainly: +/- 25%

[1] AoeL-Stellungnahme zu Kontaminationen mit Phosphorwasserstoffen im Spurenbereich in ökologischen Lebensmitteln
[2] Acid Distillation Method for Phosphin in stored Foodstuffs (Nowicki, 1978; Scudamore and Goodship, 1986)

Keywords: *Phosphine, organic food*

R-6
PESTICIDE RESIDUES IN BABY FOOD PRODUCED BY DOMESTIC MANUFACTURES OF THE REPUBLIC OF SERBIA 2010/2013

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At present, the pesticides are widely used in fruit and vegetables production and their excessive use may be harmful to baby's health so the content of their residues needs to be controlled. The baby food Directive 2003/13 established maximum residue levels (MRLs) at 0.01 mg/kg or less for all pesticides in industrially processed baby food. The enforcement of these low MRLs requires sensitive and accurate methods for analysis of pesticides in baby food. Our studies comprised the determination of the pesticide residues content in 185 baby food samples produced by domestic manufactures taken during 2010–2013 by liquid chromatography tandem mass spectrometry (LC–MS/MS) with electrospray ionization (ESI). The samples were tested regarding the content of 55 pesticides. The preparation and clean-up samples were based-on QuEChERS method. The validation study was performed according to the European SANCO guidelines 12495/2011. Analytical characteristics evaluated were linearity, LOD, LOQ, recovery and repeatability. Linearity of the method was investigated in the range from 5 to 250 µg/kg. Matrix effect on linearity and recoveries and its effects on ionization were evaluated for different matrices (apple, peach, peer, vegetable, mixture of banana and apple). In order to avoid this interference, the quantification was carried out on matrix mached calibration. The recovery was determined at three levels: 5, 10 and 50 µg/kg with mean values ranged from 69.5 to 112.0%. The repeatability of the methods was determined by spiking blank apple sample at concentration level 10 µg/kg in six replicates (RSD < 20% for all analites). The LOQ for most pesticides was 5 µg/kg. Triadimenol and pirimiphos-methyl were detected in two samples during 2010, but the detection was below MRLs. In 2011 eight samples of baby food were found to be contaminated by pesticides residues in the concentrations above MRLs (carbendazime and pyrimethanil). During 2012 all detections were below the LOQ. In 2013 for samples were with the detected carbendazime and pyrimethanil residues over MRLs. A high percentage (12.12% – for four year period, with most detected azoxystrobin, carbendazime and pyrimethanil) of positive baby food samples to the pesticide residues content is a warning that in domestic production a continuous and multilevel monitoring of food safety must be kept aimed at the successful prevention of harmful pesticide effects on infant health.

Keywords: *Pesticide residues, baby food, LC–MS/MS*

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R-7
APPLICATION OF QUECHERS AND DLLME FOR PESTICIDE AND VETERINARY DRUG RESIDUE ANALYSIS IN MILK SAMPLES BY UHPLC–MS/MS

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Currently, one of highest trade barriers that Brazil has found for the marketing of its products from the agribusiness chain is the lack of information on the presence of residues in foods produced in the country. Due to the complexity of matrices of animal origin and the low concentrations of pesticides and veterinary drugs present, there is a great need to develop efficient and reliable analytical methods for identification and quantification of residues. In this study we evaluated the linear range of standard curves, limit of detection (LOD), limit of quantification (LOQ), matrix effect and the precision and accuracy, in terms of percent recovery for, 91 pesticides and 9 veterinary drugs that were analyzed and validated by the QuEChERS method and Ultra High Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UHPLC–MS/MS) in milk samples. For validation, we performed the fortification of previously homogenised samples with solutions co ntaining the 100 compounds in three fortification levels (10, 25 and 50 μg kg^{-1}), 6 replicates for each level, and applied the extraction. The modified QuEChERS extraction method consists in the mixture of 10 mL milk with 10 mL of acetonitrile containing 1% of acetic acid (*v/v*) and shaking vigorously by hand for about 1 min. After, 4 g of anhydrous magnesium sulfate and 1.7 g anhydrous sodium acetate, were added and the agitation repeated. After centrifugation for 8 min (3,500 rpm), 4 mL of extract were transferred to another tube containing 600 mg of anhydrous magnesium sulfate and 500 mg sorbent C18, repeating the agitation and centrifugation. The extract was diluted (1:1) in the mobile phase, and then analyzed by UHPLC–MS/MS. Analytical curves prepared in solvent and in all matrix extracts showed adequate linearity between 1.0 and 250.0 μg L^{-1}, with determination coefficients greater than 0.995. The method showed satisfactory recovery values between 70 and 120% (RSD≤ 20%) to about 85% of the compounds in all matrices at levels 10, 25 and 50 μg L^{-1}. In general, the method presented LOQm value of 10 μg L^{-1}. The major disadvantage of the QuEChERS method is the low enrichment factor that can be achieved. To overcome this limitation, we proposed a DLLME after QuEChERS extraction. Milk samples were fortified at lower concentrations 5, 10 and 15 μg L^{-1}, and after the DLLME the enrichment factor was 10x. The recovery and precision results obtained with the QuEChERS-DLLME combination were between 70 and 120% (RSD≤20%). Therefore, we concluded that the proposed method can be applied efficiently for the determination of pesticide and veterinary drugs residues in milk samples.

Keywords: *QuEChERS, DLLME, food, milk*

Acknowledgement: *CAPES, CNPq, SIBRATEC*

R-8
DETERMINATION OF PESTICIDE RESIDUES IN ORANGE JUICE USING QUECHERS METHOD AND UHPLC–MS/MS

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The orange juice industry is one of the most successful sectors in the Brazilian economy. The determination of pesticide residues in food matrices is a great challenge especially because of the small quantities of analytes and large amounts of interfering substances which can be coextracted with analytes and, in most cases, adversely affect the results of an analysis. The methods of analysis of pesticide residues basically involve two steps: extraction of the compounds of interest and determination by chromatographic separation. The sample preparation step in complex matrices requires much time, and so is often the limiting factor in the analysis of pesticide residues. In this study, it was compared different extraction procedures based on the QuEChERS method for the determination of 76 pesticides in integral orange juice by Ultra High Performance Liquid Chromatography coupled to tandem Mass Spectrometry. After choosing the best preliminary test it was carri ed out the experimental design to optimize the same. The parameters evaluated for the validation were: linearity of the analytical curves, matrix effect, limit of detection (LOD), limit of quantification (LOQ), as well as precision and accuracy. The results of the validation were satisfactory, since the method presented recoveries between 70.5 to 117.5% and RSD lower than 19.2%. The method LOD and LOQ ranged from 0.1 to 12.4 μg L^{-1} and from 4.9 to 26 μg L^{-1} respectively, only acephate and deltamethrin compounds were not quantified. Thus, the method was validated for 74 pesticides in integral orange juice. The method developed was adequate for the analysis of pesticide residues in integral orange juice, since all parameters of validation were in according to the guidelines and the sensitivity achieved meets the maximum residue levels (MRLs) established by regulation for food monitoring programs. The results of this study allow concluding that the combination of QuEChERS met hod and UHPLC–MS/MS is excellent for analysis of pesticide residues due to its quickness and effectiveness.

Keywords: *UHPLC–MS/MS, QuEChERS, residues, juice*

Acknowledgement: *CAPES, CNPq, SIBRATEC*

R-9
INVESTIGATION OF MATRIX COMPOUNDS RESPONSIBLE FOR ENHANCED DEGRADATION OF ORGANOCHLORINE, ORGANOPHOSPHORUS, ORGANONITROGEN, AND CARBAMATE PESTICIDES DURING HOT GC SPLITLESS INJECTION OF QUECHERS EXTRACTS OF CANOLA SEED

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We noticed low response factors for select pesticides with hot splitless injection GC of EN QuEChERS extracts of canola seeds compared to those same pesticides in EN QuEChERS extracts of tobacco. An experiment was designed to quantify the differences by GC analyzing pesticide-spiked tobacco and canola extracts, and acetonitrile solvent-only standards. The pesticide concentrations were relatively high at 5 ng/µL to minimize any matrix-enhanced response effect, especially for the solvent-only standards. A 4 mm ID single taper with wool liner was used for the work at 250°C. Organochlorine pesticides that showed markedly low response factors when in canola seed extracts included chlorothalonil, delta-BHC, and endosulfan sulfate. Interestingly, alpha- and gamma-BHCs, and endosulfans I and II were not impacted. Other pesticides that had low responses in canola extracts versus tobacco extracts and solvent-only standards were carbaryl, methiocarb, dichlofluanid, captan, folpet, deltamethrin, and more. LC–MS/MS analysis of canola and tobacco extracts, and solvent-only standards, gave essentially the same response factors for all pesticides independent of the matrix, which proves that the low GC response factors were due to the canola extract. One theory is that isothiocyanates in the extracts from canola are leading to pesticide degradation during hot splitless injection. It is possible, too, that this effect is enhanced by having a wool-packed GC inlet liner. Fractions of canola seed extract resulting from different SPE experiments were collected fortified with pesticides and tested the same as previous canola seed extracts. This approach was useful in narrowing the matrix compounds potentially responsible for the degradation observed earlier. Fraction samples that showed the most severe pesticide degradation were characterized by TOFMS and matrix compounds with the potential to cause degradation of specific pesticides during a hot splitless GC injection were identified.

Keywords: *Pesticide degradation, GC–TOFMS, canola seed, matrix reactions*

R-10
MULTI-RESIDUE PESTICIDE ANALYSIS IN HERBAL TEAS USING THE QUECHERS EXTRACTION, CARTRIDGE SOLID PHASE EXTRACTION CLEANUP, AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY

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Herbal tea, a non-caffeinated drink made from plants, herbs, or spices, has been used throughout history for its potential medicinal benefit. Used frequently in traditional Chinese medicine (TCM), different blends of herbal material will be formulated depending on the desired medicinal properties. As with any plant-based commodity, there is the potential for pesticide residues to remain in the final product. Dried plant material found in herbal tea poses a significant challenge to the analytical chemist to detect trace levels of pesticide residues. The extract, even after an extensive cleanup, can contain a large amount of coextractive material that can completely overwhelm the target pesticides, making trace detection very difficult. Furthermore, nonvolatile materials not removed during extract cleanup deposit onto the inlet and column, requiring more frequent maintenance to be performed. We employed the QuEChERS methodology for a quick extraction of store-bought herbal teas and a combination solid phase extraction (cSPE) cartridge cleanup containing 500 mg carbon and 500 mg primary secondary amine (PSA). The percent recoveries of spiked pesticide standards and quantification of incurred pesticides were determined using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC–TOFMS).

Keywords: *Herbal teas, pesticide residues, QuEChERS, SPE, GC×GC*

R-11
MITIGATING MATRIX EFFECTS: EXAMINATION OF DILUTION, QUECHERS AND CALIBRATION STRATEGIES FOR ELECTROSPRAY LC–MS/MS ANALYSIS OF PESTICIDE RESIDUES IN DIVERSE FOOD TYPES

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Liquid chromatography tandem mass spectrometry (LC–MS/MS) is popular for food pesticide residue monitoring. Increased selectivity and sensitivity of LC–MS/MS have impacted how multiresidue methods are performed sometimes decreasing the need for rigorous sample preparation. However, this technique suffers from matrix effects causing poor data quality and difficult quantification. Matrix effects can be mitigated by sample preparation that reduces the concentration of coextracted matrix material or by experimental strategies like matrix-matched calibration that compensated for matrix effects. We considered these two aspects of multi-residue methods, sample preparation and calibration strategies, to determine recommendations considering both data quality and time and financial investments. We performed matrix effects studies and investigated two approaches for reducing matrix interferences, QuEChERS and dilute and shoot techniques, in combination with the compensation strategy of matrix-matched calibration which was compared to solvent calibration. There are compromises with each method regarding time and financial resources. A variety of food types were tested including high water (celery), high pigment (kale), high fat (avocado), citrus (lime) and dry (brown rice flour) foods, with subsequent pesticide determinations by LC–MS/MS. Samples were fortified at high and low ppb levels with over 100 pesticides representing multiple classes. We determined that with the easiest commodities, the dilute-and-shoot method and solvent-only calibration gave acceptable recovery values. However, for other commodities either a matrix-matched curve or cleanup was needed to obtain good recovery values. The high carbohydrate and citrus commodities proved to be too difficult with the specific methods we tested here. In almost every case, use of a matrix-matched calibration provided improvement.

Keywords: *QuEChERS, matrix effects, pesticide residues, LC–MS/MS, calibration*

R-12
PRACTICAL APPROACH FOR USING ANALYTE PROTECTANT IN ROUTINE ANALYSIS

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Traditionally, matrix-matched calibration standards have been employed to compensate for matrix-induced response enhancement effect which often occurs in GC analysis. Nevertheless, there are some limitations for real sample analysis: e.g. the availability of a residue-free sample and the commodity-dependent behavior of sample matrices which can lead to instability of results. Analyte protectant (AP) is a promising option for solving these matrix difficulties. Similar to matrix behavior, AP works as a masking agent to block active sites in the GC system and maintain the benefit of increasing sensitivity while overcoming the matrix effect. In all previous works, AP was used by directly adding into every analyzed extracts. This method of AP use is quite impractical for routine analysis because it is time-consuming and creates additional cost due to the large volume of AP required. Therefore, a new practical-matched approach for AP was investigated. AP solution was simply injected at the beginning of the sequence analysis as a priming agent in the GC system; sample matrices were then analyzed consecutively. QuEChERS methodology and GC–MS/MS analysis were employed for evaluation of results. The performance of AP in the priming system was tested at the default level of EU maximum residue level (MRL) for pesticide analysis. Signals of matrix standards were measured with and without AP priming to compare the consistency of results. In add ition, long-term performance of AP priming was investigated with the 50 continuous injections of matrix standards to simulate routine work. Compared to sequence analysis of sample matrices without AP priming, a significant decrease of relative standard deviation when using AP priming within a long sequence of injections clearly demonstrate the potential utilization of AP priming in real-life applications for routine analysis.

Keywords: *Analyte protectant, Matrix effect, Pesticide residues, GC–MS/MS*

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R-13
COLLISION CROSS SECTION A NEW IDENTIFICATION POINT FOR A "CATCH ALL" NON TARGETED SCREENING APPROACH

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The SANCO/12495/2011guidance document implemented in 2012 describes the method validation and analytical quality control requirements to support the validity of data used for checking compliance with maximum residue limits, enforcement actions, or assessment of consumer exposure to pesticides in the EU. For high resolution MS systems such as TOF (time of flight), the requirements for identification are that the quasi molecular ion and at least one fragment ion are obtained with Mass accuracy < 5 ppm. In addition isotope ratios and S/N are considered. It is also stated that "identification relies on proper selection of diagnostic ions" The ratio of the chromatographic retention time of the analyte to that of a suitable internal standard, i.e. the relative retention time of the analyte, should correspond to that of the calibration solution with a tolerance of ±2.5% for LC. It is also stated that "Different types and modes of mass spectrometric detect ors provide different degrees of selectivity, which relates to the confidence in identification" and requirements for identification are given which should be regarded as a guidance criteria for identification, not as absolute criteria to prove presence or absence of a compound. Current guideline strategies take an approach of making retention time tolerances more stringent and result in system specific assay parameters. Retention time tolerances are impacted upon by matrix shifts and there is a drive towards 0.1minute retention time tolerances. This approach becomes user system specific. Applying a "catch all approach" will enable a generic set of parameters to be utilised within inter/intra laboratory studies enabling screening to be performed rapidly. Additional identification points will enable less stringent screening parameters and increased specificity simultaneously. The feasibility of UPLC HDMSE has been explored. Data was initially acquired for a series of solvent standard mixtures. Subsequently the corresponding set of data was acquired for the pear, ginger, leek, mandarin matched matrix calibration series and then EU-RL proficiency test samples. The CCS values generated from the solvent standards and matrix matched calibrants were shown to statistically belong to the same population. Hence it can be shown that the CCS of the pesticide standards is independent of the matrix and can be utilized as a confirmatory parameter to increase confidence in identification and further reduce false positive and negative identifications. The use of CCS offers the potential to reduce the initial specificity of applied screening parameters. The CCS data generated was entered into a scientific library within a new scientific information system. This allowed the expected and determined CCS values to be utilized to reduce false identifications in t he proficiency test samples and matrix matched calibrant series analysed, whilst applying wider screening tolerance parameters

Keywords: *Ion Mobility, Identification Point, Collision Cross Section, Accurate Mass*

R-14
VALIDATION OF A GC–SIM–MS METHOD FOR THE DETERMINATION OF DITHIOCARBAMATE FUNGICIDE RESIDUES IN FRUITS AND VEGETABLES

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In Costa Rica, agricultural production uses high amounts of pesticides per cropped hectare. One of the most used groups are dithiocarbamates (DTC), due to their characteristics as multisite, broad spectrum, protectant fungicides. DTCs are used in Costa Rica in a wide range of crops such as banana, many vegetables and ornamental plants. Mancozeb is the most applied DTC representing more than 40% of the total pesticide imported in 2009 [1]. Other DTC fungicides commonly used in Costa Rica according to imported quantities are: propineb, metiram, ziram, zineb, thiram, ferbam and maneb. The traditional analytical method used to determine residues of DTC is an indirect method that spectro-photometrically measures carbon disulfide (CS_2), as a complex, after a hydrolysis with hydrochloric acid and subsequent distillation. It is a time consuming method, with low sensitivity and has many inconveniences related to the glassware assembly needed. Due to the urgent need in the country to analyze residues of DTC, a reliable and fast method from the EU Reference Laboratories for Residues of Pesticides [2] was tested with some modifications in typical matrices to test its suitability for routine analysis in Costa Rica and to compare it with the traditional method. With this EU method, DTCs are converted to CS_2 by a reaction with tin(II)-chloride in aqueous HCl (1:1), at 80°C. The CS_2 gas is absorbed in isooctane and its concentration measured by GC–ECD, whereas in our laboratory a GC–SIM–MS system was used for the detection. The method showed a good linearity (r^2 > 0.99), with a limit of detection (LOD) and a limit of quantification (LOQ) of 30 and 50 µg/kg respectively. The recoveries at a spiking level of 50 µg/kg of ferbam (measure as CS2) in tomato, pineapple and vegetable pear (chayote) were 118% (±10), 98% (±5) and 79% (±10) respectively (n=8). Recoveries at 500 and 5000 µg/kg levels were between 60 and 120% with a standard deviation ≤ 20%. The newly applied method shows better precision and accuracy, with a lower LOD for DTC with less time needed per sample and using lower input of chemicals, compared to the traditional method.

[1] Vega Z., E.J. 2012. Importaciones de plaguicidas durante el período de 2006 al 2009 en Costa Rica y diseño de un programa de cómputo para inclusión de datos y consulta de plaguicidas registrados en el Servicio Fitosanitario del Estado-MAG. Tesis Lic. Ing. Agr. Universidad de Costa Rica, Fac. Agr.

[2] Community Reference Laboratory for the Single Residue Methods, CVUA Stuttgart, Germany. 2009. Analysis of dithiocarbamate residues in foods of plant origin involving cleavage into carbon disulfide, partitioning into isooctane and determinative analysis by GC-ECD, Version 2, http://www.crl-pesticides.eu/library/docs/srm/meth_DithiocarbamatesCs2_EurlSrm.PDF [Accessed: 30.08.2013]

Keywords: *Dithiocarbamate fungicides, ferbam, GC-SIM-MS, residue analysis, method validation*

R-15
EVALUATION OF THE BEHAVIOR OF DIFFERENT DISPERSIVE SOLID PHASES IN ANALYSIS OF PESTICIDES RESIDUES IN TOMATOES CONSUMED IN URUGUAY

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QuEChERS methodology, has been in the last decade, a very useful tool since implies a minor use of solvents as well as less time of processing the samples, in comparison with other existing methodologies, and therefore it generates major productivity in control laboratories of pesticides residues. However, above mentioned method and its high productivity, contrasts with an inefficient clean-up in cases of some matrixes with solid phases, which are many times recommended by the manufacturers of the QuEChERS kits. Mentioned failing in the cleanliness of the matrix co-extractives, generates higher entrance of organic substances to the chromatographic system, which leads to major times of stop for the maintenance of the analytical equipment, entailing therefore, higher costs for the laboratory that processes thousands of samples per year. In this actual work, it was evaluated the behavior of three combinations of dispersive solid phases: PSA; GCB + PSA and GCB+P SA+Tolueno, for the analysis of chlorothalonil and chlorpyrifos in tomato matrix (Solanum lycopersicum). There were used tomato samples certified as organic products. The extraction was carried out by means of acetic acid 1% in acetonitrile (v/v). The detection and quantification of pesticides was done by means of GC/MSD. It was measured the absorbance of the extracts after the clean-up in a spectrophotometer UV-Visible. The three methods that were studied showed a well marked matrix effect, for the chlorothalonil it was observed a suppression of the signal, whereas in the chlorpyrifos there was an increased of the chromatographic signal for three tested methodologies. A major recovery was obtained for the PSA phase for both pesticides, at MRL´s level, being 98% for chlorothalonil and 122% for chlorpyrifos, with a CV of 9% for both pesticides. The combination PSA+GCB was the one that presented the minor recovery and precision, being the percentage recovered of 16% for chlor othalonil and 34% of chlorpyrifos.In case of the phase PSA+GCB+Tolueno, recoveries were obtained over 60 %, minor to the PSA, but the absorptions measured in the spectrum UV-visible indicate a clear decrease in the concentration of co-extractives in comparison to PSA. According to what was observed, the phase PSA+GCB is not the appropriated one for being used with this combination matrix-pesticide, due to its low recovery. Meanwhile, the other two dispersive solid phases that were studied, present recoveries between acceptable and very good. When making choice for one of these two studied methods, especially in laboratories that do not have wide availability in analytical equipments and prompt service response, other points should be taken into account, not only the recovery, but also the costs of maintenance of the analytical instruments and its repercussion in the productivity of the control laboratory. Each laboratory should evaluate their cost/benefit at the moment of se lecting the methodology.

Keywords: *QuEChERS Clean-Up recovery*

Acknowledgement: *International Atomic Energy Agency (IAEA)*

R-16
METHOD DEVELOPMENT AND VALIDATION OF ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS IN FISHES

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It is very important to analyze contaminants in fishes nowadays because of its exposure to environmental contaminations and it has the capability to enhance the contaminants in food chain. This way, using the QuEChER'sapproach, a multiresidue method was developed to analyze organochlorine pesticides and polychlorinated biphenyls in fish. On developing the method, it was initially elaboratedthe Ishikawa diagram to identify the key parameters of the proposed method which could influence the results of recovery. With these parameters we designed a complete planning with three central points to observe the standard deviation. The results showed the best extraction conditions to be applied during the validation process. The validation process used as the basis of the criteria SANCO/12495, which were evaluated from a fully automated spreadsheet to perform the calculations and evaluation criteria. With the validation data in hand, and utilizing the"validation-based" approach, it was also possible to estimate and evaluate the uncertainties of the proposed method. The method developed has met all criteria's of validation protocol adopted, and it was put into routine analysis of these contaminants in the National Agricultural Laboratory of São Paulo (Lanagro-SP). In addition, calculation spreadsheets have been generated which can be applied to future validations that need to be evaluated by the same validation protocol. Lanagro-SP is an official Laboratory of Ministery of Agriculture, Livestock and Food Supply and meets the NRCP - National Residue Control in food.

Keywords: *Pesticides, food control, validation, uncertainty measurement*

Acknowledgement: *This research was supported by CNPq and Ministery of Agriculture, Livestock and Food Supply*

R-17
PESTICIDE ANALYSIS USING AUTOMATED QUECHERS: THE DETERMINATION OF PESTICIDES IN CEREAL GRAINS

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The QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe) sample extraction method was developed for the determination of pesticide residues in agricultural commodities. Since its development, QuEChERS has been modified to accommodate other matrices such as cereal grains. The rise in popularity and ease of the QuEChERS has driven the need for automation for this manual extraction technique. By using the AutoMate-Q40, it streamlines the two part QuEChERS method from the liquid extraction to the cleanup step.
The aim of this project is to evaluate the performance and versatility of the AutoMate-Q40. Liquid Chromatography coupled to a triple-quadrupole mass spectrometry (LC/QQQ) was employed for the analysis of the LC-amenable pesticides in grains. Quantification was based on matrix-matched calibration curves with the use of internal standard to ensure method accuracy. By using the AutoMate-Q40 to streamline the QuEChERS method to cereal grains this provides us with good analytical results falling in the method guidelines (range of 70-120% and RSD <20%) for the majority of the target compounds.

Keywords: *Pesticides, Residues, Automation*

R-18
DISCOVERY OF PESTICIDE PROTOMERS USING ROUTINE ION MOBILITY SCREENING

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Criteria to instill confidence in identification include acceptable product ion ratio tolerances and relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense (abundant) ion or product ion, which should correspond to those of the calibration standard at comparable concentrations and measured under the same conditions. It is known that ion ratio performance can vary with instrumentation and can also be affected by sample concentration and matrix. The SANCO/12495/2011guidance document implemented in 2012 describes the method validation and analytical quality control requirements to support the validity of data used for checking compliance with maximum residue limits, enforcement actions, or assessment of consumer exposure to pesticides in the EU. Here we explore the use of high definition mass spectrometry (HDMS) as an important tool to gain a greater understanding of variation in ion ratios. This technique offers some unique advantages to profiling complex matrices. It uses a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility spectrometry (IMS) is a rapid, orthogonal, gas phase separation technique which allows another dimension of separation to be obtained within an LC timeframe. Compounds can be differentiated based on size, shape and charge. In addition, both precursor ion and fragment ion information can be acquired in a single injection in an HDMS experiment referred to as HDMSE. Empirically isobaric pesticide protomers have been identified and characterised using ion mobility. It has been possible to separate the protomers (ions different only by their protonation site), determine their respective collision cross section and individual protomer fragmentation dissociation pathways. This has enabled unique visibility of product ion formation information, enabling the product ions to be selected that will result in improved product ion ratio reproducibility. For the assay, UPLC–HDMSE experiments were performed on a Synapt G2-S using a series of standard solutions, spiked matrices and a previous proficiency test.

Keywords: *Protomers, Ion Ratios, Ion Mobility, Collision cross section*

R-19
IMPROVEMENT OF PESTICIDE RESIDUE ANALYTICAL METHOD FOR REMOVING IMPURITIES IN ALLIUM CROPS

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In order to find the proper reduction method of the impurities produced by alliinase activity in Allium crops (garlic, onion, shallot and welsh onion etc.), the analytical method for pesticide residues using HPLC–UVD(DAD) was developed by adjusting pH and temperature. In pH control, removal rate of impurities were showed 81.8% and 68.4% in strong acid (pH 2) and strong alkali (pH 12) condition respectively. But this method was inappropriate for some pesticides because 29 pesticides including thiophanete-methyl in acid, 28 pesticides including ethaboxam in alkali were decomposed. In temperature control, sample was treated by cold storage before sample preparation. Removal efficiency of the frozen samples (at -20°C) were founded 68.7~76.1% in welsh onion and 66.8~77.8% in shallot, respectively. But it was consumed a lot of time for freezing. Therefore, mixing with dry ice to sample was selected for the proper method to keep low temperature during analysis. Mixing ratio between dry ice and sample for reliable result was appeared 0.5~1.0 (dry ice/sample, w/w) Using dry ice method was achieved 87.2% of removal efficiency in shallot, but the efficiency was getting decreased 48.1% in 2 hrs later. Recoveries were satisfied in 70~120% of analytical guideline in almost pesticides. Comparing with existing pesticide residue method, the improved method was shown the high removal efficiency in shallot of 87.2%, Welsh onion of 93.7%, garlic of 46.3% and onion of 66.5%.

Keywords: *Pesticide Residue, Dry ice, Removing Impurities, Allium Crops, Acid-alkali*

R-20
DETERMINATION OF CHLORMEQUAT RESIDUES IN MILK BY UPLC–MS/MS

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Chlormequat is a plant growth regulator generally used to promote lateral branching and flowering of ornamental plants. It is also frequently used to improve yields for different types of cereals in Europe. These cereals are often processed into animal feed. Consequently chlormequat residues could potentially be found in matrices of animal origin. The MRL in milk were fixed at 0.05 mg/kg in annex I part B of the regulation 396/2005. Milk is a very complex matrix composed of different proteins and fat. Therefore, intensive clean-up is needed before injection to prevent interference or clogging during LC analysis. For the extraction, 4ml of water is added to 1g of milk just after the addition of chlormequat D4 as internal standard. The clean-up strategy selected was the use of dichloromethane and acetic acid to eliminate fat and precipitate protein, respectively. This step was followed by a solid phase extraction (SPE) of the aqueous phase on a Weak Cation Exchange cartridge. The chlormequat was eluted by using ammonium acetate, before injection on an UPLC–MS/MS system (Waters premier). Due to his ionic characteristic, no retention is observed on classical C18 column. The reverse phase polymethacrylate Shodex RSpak DE413 (150 × 4.6 mm) recommended in the CEN method prEN15054 has been selected. Isocratic condition (water/acetonitrile/ methanol/acetic acid – 71.25+17.5+7.5+3.75 V+V+V+V 50 mM ammonium acetate are used at a flow of 0.4 ml/min. The observed a retention time is 4.0 min. The analytical method has a limit of quantification of 0.025 mg/kg (limit of detection of 0.0125 mg/kg) and was validated following SANCO/12495/2011 criteria at the LOQ and at a higher level (10 times LOQ – 0.25 mg/kg). Results show a recovery of approximately 100% (between 100.6 and 103.0) with a repeatability and in-house reproducibility below 10%. These very good validation data are correlated to the robustness of the method and to the use of an isotopic surrogate.

Keywords: *Chlormequat, milk, extraction, clean-up, LC~MS/MS*

R-21
A COMPARISON OF PESTICIDE RESIDUES SPIKING VALUES AND CONSENSUS ASSIGNED VALUES FROM PROFICIENCY TESTING SAMPLES

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When determining the value of an analyte in a proficiency testing sample, there are three estimates to consider. These are *(i)* the spike level, *(ii)* the homogeneity mean, and (iii) the consensus assigned value, which is based upon results submitted by participants. Here we examine the difference between these three values, and evaluate which is the most appropriate when setting the assigned value for pesticide residues analysis in FAPAS proficiency test samples. Proficiency testing data from FAPAS' complete pesticide residue analysis range were collated and examined. The statistical analysis used the assigned value as a reference value for two-way comparisons with homogeneity means and spiking values. The results showed that the assigned value and homogeneity mean were consistently in good agreement; however, the spiking value was systematically a factor of 1.22 higher than the consensus assigned value. The discrepancy did not appear to be a result of analyte concentration, nor the chemistry of the analyte. However, some variance was able to be attributed to factors derived from the specific Series and specific rounds within a Series, as well as variance within a round. Here we try to identify the cause of this variance, considering *(i)* impure spikes, *(ii)* overestimated recoveries, or *(iii)* loss of spike during preparation, storage or distribution.

Keywords: *FAPAS, Proficiency Testing, Pesticide Residues*

R-22
ENHANCING GENERAL UNKNOWN SCREENING WITH DATA INDEPENDENT ANALYSIS ON A QUADRUPOLE ORBITRAP MASS SPECTROMETRY SYSTEM

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In residue analysis of environmental and food samples the need exists for the analysis of larger sets of analytes at the same time. Recent developments in scan speed of triple quadrupole mass spectrometry systems have fuelled this development, but even more the steadily increasing use of high resolution accurate mass (HRAM) mass spectrometry instrumentation (mainly TOF and Orbitrap based instruments) in residue analysis leads to numerous new options in this field as well into new challenges. One of these challenges is the confirmation step for the analytes, since precursor ion selected fragmentation comes to its technical limits in terms of the number of analytes and becomes impossible in case of analytes yet unknown or disregarded. The alternative of fragmentation without precursor ion selection (as "All Ion Fragmentation" or "MSE") has proven to be very powerful but still faces some limitations in sensitivity of fragments for low concentrated compo nents. The Thermo Scientific Q Exactive mass spectrometry system now offers an alternative, filling the gap between precursor ion selected data dependent MS2 (ddMS2) and All Ion Fragentation (AIF). In the Data Independent Acquisition (DIA) scan mode, the full scan mass range is divided into smaller ranges, typically in the range of 20 to 50 Daltons. This dramatically enhances the dynamic range for the fragment scans, resulting in significant higher sensitivity of the significant fragments needed for compound confirmation. At the same time all options for suspect screening or even General Unknown Screening remain fully available as in AIF. In this study we show an example of environmental analysis directly comparing DIA directly to ddMS2 and AIF.

Keywords: *Data Independent Acquisition, HRAM, Orbitrap, General Unknown Screening*

R-23
EVALUATION OF HIGH RESOLUTION LC-MS ORBITRAP AND GC–MS FOR THE DETERMINATION OF ENDOCRINE DISRUPTOR PESTICIDES IN FOOD SAMPLES IN EPIRUS REGION MARKET (NW GREECE)

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In recent years effects have been reported in animal species and human beings that are attributed to the influence of certain substances on hormonal systems. Endocrine disrupting compounds (EDCs) are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects. Food is likely to be one of the most important routes of human exposure to EDCs. Therefore, an urgent demand appears for the development of analytical methods to monitor Endocrine Disrupting Compounds (EDCs) in food so that regulatory limits may be enforced. In the present study endocrine disruptor pesticides belonging to different chemical classes were analyzed in fruits and vegetables from the region of Epirus (NW Greece) by two extraction methods and two chromatographic techniques for the determination of pesticide residues. A UHPLC–HR Orbitrap Mass Spectrometer technique and gas chromatography-mass spectrometry (GC–MS) were applied in the detection of 13 endocrine disrupting compounds (Trifluralin, Dimethoate, Lindane, Vinclozolin, Metribuzin, Heptachlor, Fenitrothion, Malathion, Chloropyrifos, Pendimethalin, Procymidone, Prochloraz, and Deltamethrin) For UHPLC–HR Orbitrap MS extraction method was evaluated using the acetonitrile-based QuEChERS sample preparation technique, followed the standard approach. For GC–MS technique Ultrasound assisted- emulsification microextraction (USAEME) was employed for for the extraction of target analytes from food commodities. The proposed method (USAEME) was validated according to the guidelines included in the SANCO/10684/2009 document and provided high selectivity, enrichment and reproducibility. To verify the confidence in the comparability of the results between LC and GC analysis, a statistical comparison was performed. Equivalence was evaluated by applying the F-test for equality of the variance and the T-test for equality of the mean measured concentration among the seven measurements of each sample. Neither the individual F-test nor the T-test revealed significant differences, indicating the equivalency of the methods, provided that the analyte concentrations lie within the dynamic measuring range of each detector. The proposed method has been applied for the determination of the target EDCs in fruit and vegetables samples taken from traditional and local markets from the region of Epirus (NW Greece).

Keywords: *Pesticides, EDCs, Orbitrap, GC-MS, fruits and vegetables*

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R-24
AN INVESTIGATION OF RESIDUAL CHARACTERISTICS OF QUINTOZENE(PENTACHLORONITROBENZENE) IN GINSENG

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Ginseng (*Panax ginseng C. A. Meyer*) is one of typical medical plants with fleshy roots. It is usually grown in shading condition for a long period lasting up to 4-6 years so that its disease tolerance is lower. For this reason, pesticides are frequently applied to protect ginseng from diseases. Quintozene (Pentachloronitrobenzene; PCNB) was originally synthesized in 1868 and have been used as excellent fungicides for ginseng seeds and soil, such as *Rhizoctonia solan*i and *Sclerotium rolfsii*, to mainly prevent crop from damping-off. Quintozene generates approximately 20 different types of metabolites in the process of metabolism, due to various environmental factors including sunlight and soil. The standards for its maximum residue limits prescribe that Quintozene should be applied by adding up the concentration of three metabolites such as Quintozene ("PCNB") as a parent compound, Pentachlorothioanisole ("PCTA") and Pentachloroaniline ("PCA") as metabolites. The standard should be 0.1 mg/kg for ginseng. In Korea, Quintozene was used for the first time in 1969, and then its use was prohibited in 1987, due to its residue in environment. Nevertheless, residues of Quintozene have been continuously found in ginseng. In this study, therefore, it was applied twice to ginseng cultivating field in order to examine its residual characteristics. The first application of pesticide to soil was performed in April. The average weight of each ginseng root was 30g in April and 45g in October, respectively, which showed about 50% increase in weight. On the contrary, 0.130 mg/kg of Quintozene was detected in ginseng in the beginning, and then its concentration continuously increased and amounted to 0.236 mg/kg in the harvest season, which showed 81.5% increase. It seems that the reason is that an increase in concentration occurs fasten than the reduction of pesticide, due to ginseng's strong absorption. The residual rate of Quintozene as a parent compound and PCTA and PCA as metabolites showed that PCTA and PCA increased to 21.3% and 17.8% in October, respectively, while Q slowly decreased to 69% and its half-life was 347 days. The second application of pesticide to the ginseng steam was carried out in August. While the treatment plot of the second application had a similar shape to the first application, the residual concentration of the second treatment plot was 1.621 mg/kg which was more than two times higher than the first treatment plot. Over time, the concentration in the area of rhizome was 11.172 mg/kg which was approximately 7 times higher than that of all ginseng. It seems that the reason is that pesticides were sprayed during a period of time when stems and leaves were very long and dense and that eventually pesticides were accumulated in the area of rhizome.

Keywords: *Ginseng, Quintozene, Pentachlorothioanisole, Pentachloroaniline, metabolites*

R-25
ANALYSIS OF PESTICIDES IN TOMATO, PEPPER, ORANGE AND GREEN TEA EXTRACTS USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO QEXACTIVE

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High resolution mass spectrometry is a technique which helps to overcome some typical limitations of triple quadrupole mass spectrometers. High resolution instruments provide accurate mass measurement. Spectrometers working in full scan mode can register unlimited number of compounds. This feature allows to perform non-target analysis and retrospective analysis. Moreover HRMS saves time because optimisation of acquisition parameters for each compound is not required. Hybrid instruments are also equipped in quadrupole mass filter and collision cell. Thanks to this it is possible to perform MS/MS experiments [1].Orbitrap is a high resolution mass analyser. This analyser is build from two curved electrodes: central electrode (which is sustained at high voltage) and outer electrode (which surrounds central electrode). Ions are injected into the Orbitrap in small packets. Strong electrical field inside the trap pushes them towards the equator thus initiating axial oscillations, while rotation around the central electrode keeps ions from falling onto the central electrode. Axial oscillations are detected and after that time-domain signal is converted into a frequency and then into m/z spectrum by Fourier transform [2]. Blank extracts of four commodities were prepared according to QuEChERS protocol. Selected matrices represented different levels of complexity. Chosen matrices were tomato (the cleanest extract, the easiest analysis), pepper, orange and green tea (the dirtiest extract, the most difficult analysis). The extracts were evaporated and redissolved in standard solutions of pesticides to obtain concentrations of 10, 50, 100 and 500 ppb. Before analysis extracts were diluted five times with ultra pure water. Extracts were analysed with UPLC coupled to QExactive (hybrid of quadrupole and Orbitrap) working in full scan mode. Obtained results were used to evaluate linearity, repeatability, limit of detection and matrix effects. All experiments were carried out in three resolutions: 17,500, 35,000 and 70,000 FWHM (full-width at half-maximum peak height at *m/z* 200). During data treatment mass tolerance was set at 5 ppm. To fully use potential of QExactive also simultaneous MS and MS/MS experiments were carried out. In data-depending-acquisition extracts were analysed in full scan mode. However, when parent ion from the target list was detected, instrument instantly was changing working mode to MS/MS and fragment ions were registered. Carried out experiments demonstrated usefulness of QExactive instrument in pesticide analysis in fruit, vegetables and tea. Orbitrap technology assured enough sensitivity to detect most of analysed pesticides at level 10ppb. Improvement of sensitivity, linear range, repeatability and peak shape was noticed when higher resolutions were applied.

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Keywords: *Pesticides, Orbitrap, QExactive*

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R-26
ELIMINATION OF MATRIX EFFECTS AND INTERFERENCES WHEN PERFORMING HIGH SENSITIVITY AND HIGH SELECTIVITY LC–MS/MS SCREENING

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Recent regulations on food analysis require the screening for pesticides using confirmatory techniques, such as GC–MS(/MS) and LC-MS/MS. With more than 1000 pesticides of more than 100 compound classes there is a demand for powerful and rapid analytical methods, which can detect very low concentrations in food matrices. Matrix effects, like ion suppression and ion enhancement are a continuous challenge for food testing laboratories due to the complexity and variety of food samples to be tested. Here we present a high sensitivity and high selectivity LC–MS/MS method that combines quantitation with identification based on Multiple Reaction Monitoring (MRM) and full scan MS/MS data. Food samples, including a variety of fruits and vegetables, but also tea and herbal products were extracted using a QuEChERS procedure [1] and injected into LC–MS/MS after extensive dilution to minimize or possibly eliminate matrix effects. LC separation was performed using a Shimadzu UFLCXR system with a Restek Ultra Aqueous C18 (100 × 2.1mm. 3 µm) column and a gradient of water and methanol and ammonium formate buffer with a total run time of less than 20 min. Detection was performed on the new AB SCIEX QTRAP® 6500 system using Electrospray Ionization. In addition, SelexION technology was used differential mobility separation (DMS) to enhanced resolving power prior MS/MS detection to remove matrix interferences [2]. Targeted pesticides were quantified and identified using a Scheduled MRM method for best accuracy and reproducibility. The superior sensitivity of the MS/MS detector was used to dilute sample extracts extensively (up to 1000×) to completely eliminate matrix effects in most cases. DMS was used as an additional tool to remove matrix interferences when detecting tricky to analyze (small molecular weight and high polarity analytes).

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Keywords: *Pesticides, LC-MS/MS, ion mobility, matrix effects, matrix interferences*

R-27
AUTOMATED DERIVATIZATION, SPE CLEAN-UP AND LC–MS/MS DETERMINATION OF GLYPHOSATE AND OTHERS POLAR PESTICIDES

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Glyphosate is a common broad-spectrum systemic herbicide used widely to kill weeds especially annual broadleaf weeds and grasses known to compete with crops. There is an interest in the reliable and sensitive quantitation and identification of glyphosate residues, its metabolite AMPA, and the related glufosinate in food and water. Commonly large volume injection into ion chromatography or LC systems based on HILIC followed by sensitivity MS/MS detection is used for analysis. However, interference can influence results in complex samples since the method does not use any cleanup. Derivatization techniques can be used successfully. The method presented here uses derivatization with FMOC-Cl followed by automated SPE cleanup using a Gerstel front-end and detection using LC–MS/MS with an AB SCIEX QTRAP® 4500 system. Limits of quantitation in food were found below the target 100 µg/kg allowing dilution to minimize potential ion suppression. In drinking water samples glyphosate was quantified below 0.1 µg/L. Linearity of over three orders of magnitude with r>0.999 was observed with excellent reproducibility because of the complete automation of the sample handling procedure.

Keywords: *Glyphosate, derivatization, automation, water, food*

R-28
HYPHENATION OF PLANAR CHROMATOGRAPHY TO CONTROL THE OCCURRENCE OF PESTICIDE RESIDUES IN APPLES

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Pesticides are of great importance in crop production. They are widely used to fight against pests and diseases. Therefore, day after day, pesticides control has become an important matter for food industry because there is a great deal of concern about healthy diet, especially for baby food. European guideline 76/895/EEC limits drastically pesticide residues in fruits. Anyway, new sanitary requirements are emerging since the past few years that harden the market. Not only Europeans as English, Germans or Russians, but also far-away countries as New Zealand tend to decrease pesticide residues levels in apples, sometimes leading to zero residues. Simultaneously, multiple studies are carried out in analytical chemistry to improve residues analysis in food matrixes.
In the framework of a project that aims to achieve zero residue of pesticides detectable in apples, hyphenating HPTLC with different types of spectroscopy, has been developed. Quick Easy Cheap Effective Rugged and Safe (QuEChERS) sample preparation is used to extract and purify pesticides from apple peels. Anyway, matrix effects due to apple peels are still important and have a negative impact on the limits of detection and quantification of any analytical method. HPTLC further purifies the QuEChERS extracts: a lot of matrix analytes, co-extracted with pesticides, are thus well discarded. Finally, the isolated recovered pesticides are directly analyzed by the different spectroscopic devices: mass spectrometry, infrared spectroscopy and bioautographic assays.
This study demonstrates that this method can be used to control rapidly and in an effective way the presence or absence (zero residue) of the seven targeted compounds.

Keywords: *Pesticides, apple, planar chromatography, spectroscopy*

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R-29
APPLICATION OF A PROTOTYPE MICROFLUIDIC DEVICE WITH MS FOR THE SCREENING OF PESTICIDE RESIDUES IN FOOD ANALYSES

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Full spectra acquisition and the specificity of accurate mass measurement is well characterised. It is used in combination with time tolerances, isotopic matching, fragment ions/ratios and response thresholds to help reduce false positive and false negative identifications in screening assays. Advances in mass spectrometry have vastly improved sensitivity for full spectral analysis, but further sensitivity enhancements would improve the mass spectral data quality. This is especially important to avoid compromised precursor ion or fragment ion information, and ensure high mass accuracy below the legislation levels. We will present data acquired using a prototype micro fluidic device, interfaced to a Xevo G2-S QTof mass spectrometer operating in MSE data acquisition mode. The assay is based on the analysis of sample extracts, matrix matched calibrants (pear, ginger, leek and mandarin) and quality control samples generated for an EU-RL proficiency test. These samples were analysed using a prototype ceramic microfluidic device containing a BEH C18 analytical channel (150 µm × 100mm) along with an ionisation emitter in one interchangeable device, coupled to a Q-Tof MS. All microfluidic, gas and electrical connections are automatically engaged when the ceramic microfluidic device is inserted into the source enclosure. The chromatographic gradient was provided from a nano UPLC system with all separations occurring on the prototype micro fluidic device. The results were compared to those previously obtained, where analysis was performed using conventional UPLC. Initial results have shown gains in both sensitivity and signal to noise with no compromise in the linearity correlation coefficients for the matrix matched calibrants ($r^2 \geq 0.95$). Improvements in sensitivity have enabled matrix dilution to be performed and routine detection of 1 pg on column sample loadings to be obtained.

Keywords: *Microfluidic Tile Pesticide residue matrix*

R-30
THE APPLICATION OF APGC COUPLED TO TANDEM QUADRUPOLE MS AS A COMPLETE SOLUTION FOR THE ROUTINE ANALYSIS OF PESTICIDE RESIDUES IN BLACK AND GREEN TEA SAMPLES

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Atmospheric Pressure GC (APGC) is a technique that has potential for the routine analysis of GC amenable analytes. The ionisation is a 'soft' process comparable to atmospheric pressure chemical ionisation (APCI) resulting in very low in source fragmentation of analytes. The low fragmentation rate means that both sensitivity and selectivity can be increased and in the case of multiple reaction monitoring (MRM) experiments the precursor ion selection process is simplified especially when compared to conventional Electron Ionisation (EI). Another advantage being that the APGC interface can be coupled to a mass spectrometer with an electrospray ionisation (ESI) source thus enabling a rapid changeover between GC and LC on a single instrument with no compromise in data quality. The application of pesticides during tea crop cultivation (and/or storage) represents a common practice for pest and plant disease control. Maximum residue limits (MRLs) for many pesticides have been established within the EU (Regulation (EC) No 396/2005). At present, the EU pesticides database contains a list of 448 pesticide residues with respective MRLs for tea ranging between 0.005 and 350 mg kg^{-1}. Several alerts concerning the presence of pesticide residues exceeding the MRLs in tea have been reported under the Rapid Alert System for Food and Feed (RASFF). The chemical and physical properties of pesticides may differ considerably. For example, there are several acidic pesticides whilst others are neutral or basic. A number of compounds are very volatile whilst several have very low boiling points and high polarity. This chemical diversity causes significant challenges in the development of a ''universal'' multi-residue method which should have the widest scope po ssible. For these reasons, GC–MS with electron impact (EI) ionization and the combination of LC with tandem mass spectrometers (LC–MS/MS) using ESI are identified as the techniques most often applied in multi-residue methods for pesticides in the current literature. In this study the Xevo TQ-S tandem quadrupole MS in combination with APGC and ESI ionisation was used to develop and validate a quantitative method for a selection of circa 60 LC and GC amenable pesticides applicable for the analysis of black and green teas. MRM transitions were optimised for each of the pesticides following APGC and/or UPLC separation. A generic QuEChERS extraction procedure based on the method by Cajka et al, 2012 was used. A selection of 20 different samples of leaf tea was included during the method validation process. The following parameters were determined; the Screening Detection Limits; analyte % recovery; linearity; repeatability; reproducibility and response stability. The APGC based method performance was compared to conventional GC and LC–MS/MS analysis in terms of sensitivity; ease of use; applicability for multi-residue analysis and overall performance.

Keywords: *APGC, pesticides, tea, GC–MS, LC–MS*

R-31
NON-TARGETED SCREENING DURING TARGETED PESTICIDE RESIDUE ANALYSIS IN FRUIT DRINKS USING GC–MS/MS WITH COMBINED MRM AND FULL SCAN ACQUISITION MODES

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One limitation of the classic multiple reaction monitoring (MRM) approach to pesticide analysis on GC triple quadrupole systems is that, due to its targeted nature, compounds not part of the target list are not detected. This carries a risk that non targeted contaminants can freely move through the food chain undetected, even when they are present at large concentrations. Targeted MRM approaches are chosen for pesticide residue testing as they allow a sensitive, selective and high quality analysis at the levels of interest. Full scan (FS) acquisitions, especially those using nominal mass can suffer from problems with sensitivity and selectivity in comparison to MRM. In order to start to adopt a "horizon scanning" approach in addition to regular routine target analysis on a GC triple is not practical when considering separate analyses as samples need to be injected twice. It is much more efficient switch between MRM and FS within an analytical run so that all necessary data can be acquired in one single injection. That said, if this is to be used, the analytical performance must be maintained to a level that still allows the reporting of pesticide residues to the required limits. The Thermo Scientific TSQ 8000 GC Triple Quadrupole MS was used to monitor 147 pesticides in fruit drinks at the same time as acquiring full scan data. Spectral data from FS acquisition was interrogated and screened against NIST EI libraries. Calibration curves generated from MRM data for the large majority of targets studied met a linear least squares calibration with a correlation coefficient of R^2>0.98. The low level precision of the alternating MRM/FS method resulted in comparable MDLs to that of performing a targeted analysis only. This was attributed to the efficient scheduling of SRM transitions using timed-SRM function in combination with fast full scans This first data presented here suggests that monitoring the background in routine samples can be performed practically during routine target analysis on a GC triple quadrupole instrument. This can have some interesting application in monitoring trends in samples and sample types, efficiency of sample preparati on process and provide another safety check for occurrence of potential non-targeted contaminants.

Keywords: *Pesticides, screening, triple quad, GC-MS, fruit drinks*

R-32
PREPARATION OF NATURAL INSECTICIDE EXTRACTS FROM QUASSIA AMARA

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Organic farming is a modern and increasingly common way of sustainable food production Nevertheless, application of common pesticide formulations is not allowed in organic farming and – according to the Commission regulation (EC) No. 889/2008 – the control of pests/insects can be performed using only limited number of (mostly natural) pesticides. For example, wood of Quassia amara contents bitter compounds (quassin and neoquassin) that possess an insecticidal and insect repellent effect [1]. In Germany and Switzerland, a commercial preparation with standardized quassin content is used; however, its high price is a limiting factor for a wider application. A non-standardized extract prepared by farmers directly from wood of Quassia amara is therefore a cheaper alternative [2]. Preparation of aqueous Quassia amara extracts can be carried out by various procedures that differ mainly in amount of wood chips, temperature and time of extraction. The aim of our study was to propose the most effective technique with regard to the yield of quassin and neoquassin in the final extract. The analysis of aqueous extracts (i.e. insecticidal preparation) was carried out using a liquid chromatography with a tandem mass spectrometry (LC–MS/MS). Simple method of aqueous extract preparation often used by farmers in practice was supplied by The Crop Research Institute and this method was adjusted to laboratory conditions (one-hour boiling of wood chips). Five modified procedures were tested for leaching of wood chips. Extended period of sample boiling had no significant effect on the yield of both target (active) substances in final extract and leaching of wood in cold water overnight showed results similar to the one-hour sample boiling. On the contrary, leaching of wood in lukewarm water for only one hour or dipping wood chips in hot water were shown to be less effective compared to one-hour sample boiling. Based on these results, the yield of quassin and neoquassin will be further tested in extracts prepared by two procedures that showed the highest amount of both target analytes in final extracts and are cost-effective in practise: (i) one-hour wood samples boiling and (ii) cold leaching of wood chips overnight.

[1] Sarais G., Cossu M., Vargiu S., Cabras P., Caboni P.: Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric Determination of Quassin and Neoquassin in Fruits and Vegetables. (2010) J. Agric. Food Chem., 58, 2807–2811.
[2] Psota V., Ourednickova J., Falta V.: Control of Hoplocampa testudinea using the extract from Quassia amara in organic apple growing. (2010) Hort. Sci. 37(4), 139–144.

Keywords: *Natural insecticide, Quassia amara, quassin, neoquassin, LC-MS/MS*

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R-33
THE HALF-LIVES OF BIOLOGICAL ACTIVITY OF CLOTHIANIDIN AND IMIDACLOPRID ON CHIVES

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This study was performed to investigate pre-harvest residue limit (PHRL) in chives and estimate biological half-life for residue of each pesticide. The chives were sprayed with the insecticide clothianidin and imidacloprid of standard and double application rates. Clothianidin and imidacloprid were sprayed once at 15 days before harvest on chives under greenhouse conditions. The chives of standard and double were sampled 9 times. The pesticides from chives samples were extracted with acetonitrile and analyzed by UPLC/MSMS. Their quantitation limit of Clothianidin and Imidacloprid was 0.003 mg/kg. Recoveries of Clothianidin at fortification levels of 0.01 and 0.05 mg/kg were 99.4±6.5% and 86.1±3%, respectively. Recoveries of Imidacloprid at fortification levels of 0.01 and 0.05 mg/kg were 87.8±4.6% and 95.5±2.5% respectively. The biological half-lives of Clothianidin were about 4.9 days at standard application rate, and 4.5 days at doupble application rate. The biological half-lives of Imidacloprid were about 2.1 days at standard application rate, and 2.5 days at doupble application rate.

Keywords: *Imidacloprid, Clothianidin*

R-34
EVALUATION OF QUECHERS METHOD WITH ZIRCONIUM DIOXIDE-BASED SORBENT AS DSPE CLEAN-UP FOR PESTICIDES DETERMINATION IN AVOCADO AND ALMOND

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Because of high fat content avocado and almond are considered as “difficult” matrices. The main challenge is in obtaining an extract which contains the target analytes but possibly low amount of fat. The most common methods for fat removal from extract are low temperature precipitation (freezing-out), gel permeation chromatography (GPC) and adsorption (dispersive solid-phase extraction, solid-phase extraction). The aim of the work was to validate Z-Sep® sorbent as dSPE clean-up material for QuEChERS extraction of pesticide from avocado and almond. Z-Sep® is commercial name of mixture of two sorbents, C18 and silica coated with zirconium dioxide, proportion of ZrO_2/C18 is 2/5. Samples were analysed by gas chromatography and liquid chromatography coupled with triple quadrupole mass spectrometers working in multi-reaction monitoring mode. For experiments 241 pesticides were selected, 123 were analysed by LC–MS/MS and 183 by GC–MS/MS. Validation was carried out according to DG Sanco guideline [1]. In the validation recoveries, limit of quantitation, linearity, matrix effects, as well as the inter- and intraday precision were studied. For recovery studies samples were spiked with the standard solution of pesticides in methanol. Selected spiking levels were 10 µg/kg and 50 µg/kg. Linearity of LC and GC systems were was evaluated by assessing the signal responses of the target analytes from matrix-matched calibration solutions prepared by spiking blank extracts at seven concentration levels corresponding to 1–500 µg/kg in the sample. Matrix effects were calculated from slopes of calibration curves in solvent and in matrix. In case of each pesticide the lowest fully validated level was considered as limit of quantitation. Among LC analysed pesticides, 118 (96% of total number) were successfully validated in avocado and 104 (84% of total number) in almond. In case of GC analysed pesticides 166 (91% of total number) were successfully validated in avocado and 129 (70% of total number) in almond.

[1] European Commission DG-SANCO (2012) Method validation and quality control procedures for pesticide residues analysis in food and feed. No. SANCO/12495/2011.

Keywords: *QuEChERS, Fatty matrices, Pesticides, Z-Sep, validation.*

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R-35
MMM GC_OIL: THE SIMULTANEOUS CLEAN UP AND MEASUREMENT OF 200 PESTICIDES, EPA PAHs, 18 PLASTICIZERS AND CONGENER PCBS IN FAT AND OIL SAMPLES

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The multi/multi method (MMM) is a combination of two or more multi methods in one run. It saves significant capacity in the lab and in equipment and also it reduces the production costs. Benefits for the customer are a shorter TAT and lower costs. The production lab enhances the competitive capability. In this case, we have combined the multimethod for pesticides with multimethod for PAHs, the multi method for plasticizers and PCBs in fat and oil matrices. Moreover it not only means simultaneous measurement of 238 pesticides and different contaminants, we have also adapted the clean up procedures so that we are now able to do a single sample preparation for all these substances. Our MMM has to be able to determine, whether each of the substances tested is within the legal requirements for that substance. The analytical scope includes - 200 of the most important “GC pesticides” like organochlorine and organophosphorous insecticides, pyrethroids and some others. The LOQ for most of the substances is 0.01 mg/kg, reco range: 75–125 % – 13 of the EPA PAHs (LOQs: 0.5–1.0 µg/kg) with the exception of three light PAHs naphthalene, acenaphthene and acenaphthylene, reco: 65–95 %, quantification – 18 of the most important phthalates, adipates with the LOQ of 0.05–0.2 mg/kg and Bisphenol A (LOQ 0.01 mg/kg) – 7 congenere PCBs with the LOQs 0.5–1.0 µg/kg For this MMM we used GC–MS/MS form Thermo Finnigan with the PTV injection and automatic liner exchanger from Axel Semrau. The sample preparation was made with automatic GPC form LCtech The biggest problem in cleanup was the carry over value of DBP (dibutylphthalate) and DEHP (diethylhexylphthalate). To avoid this contamination we cleaned the GPC solvents and we only used heated out glass ware and liners. In spite of that we observed strong carry over effects of the light PAHs naphthalene, acenaphthene and acenaphthylene. That’s why we excluded these substances from our method. We measured these three PAHs in the separate run. In conclusion we proved the method by measurement of proficiency test materials (FAPAS) and reanalysis of the known samples. In most cases we observed similar results.

Keywords: *Multimulti methods, pesticides, PAHs, PCBs*

R-36
SIMULTANEOUS DETERMINATION OF PESTICIDES, PHARMACEUTICALS AND MYCOTOXINS IN RICE USING LIQUID EXTRACTION FOLLOWED BY LIQUID CHROMATOGRAPHY/QUADRUPOLE-LINEAR ION TRAP MASS SPECTROMETRY

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A simple, sensitive method was developed and validated for the simultaneous analysis of 250 pesticides, 10 pharmaceuticals and 8 mycotoxins compounds in rice plants (stem/leave and grain). Stems/leaves were obtained from the Albufera Natural Park rice fields (Valencia, Spain) during the 2011, 2012 and 2013 campaigns. Grain samples were collected in 2013 (from the 2012 harvest) from rice mills of different municipalities in the province of Valencia. Individual stock solutions were prepared at concentrations of 10 µg/mL and 1 µg/mL in water/acetonitrile (ACN) 98/2 *v/v*. Approximately 5 g of each homogenised sample were weighed into a 50 mL centrifuge tube. To perform the extraction process, 20 ml of 75% (*v/v*) ACN aqueous solution with 1% (*v/v*) of formic acid were added. The sample was homogenised for 60 min and centrifuged at 4,000 rpm for 5 min. Chromatographic separation was performed with an Atlantis T3 at a flow rate of 0.3 ml min^{-1}. A programmed gradie nt was employed with the mobile phase by combining solvent A (5 mmol l−1 ammonium formiate with 0.1% (*v/v*) formic acid) and solvent B (methanol). The quantitative analysis was done by HPLC–MS/MS equipped with an electrospray ion (ESI) source. The injection volume was 5 µl in the ESI+ and 10 µl in the ESI- modes. The column temperature was kept at 40°C, the interface temperature was set at 400°C, while the source temperature was 120°C. The matrix interferences affecting accuracy and the matrix-matched calibration curves were prepared for control and quantification purposes. Blank samples were fortified by spiking with different aliquots of a standard solution mixture of standards within the 1–500 µg/L range. Linearity was studied by analysing standard solutions at five concentrations within the 1–500 µg/L range. Satisfactory linearity was assumed by using least squares regression when the correlation coefficient (r) was higher than 0.99. Accuracy and precision were s tudied by recovery experiments in rice spiked at two levels. Recoveries were generally over 65% and the limit of quantification was 1-10 µg kg^{-1}, except for aflatoxin B1 (0.3 µg kg^{-1}). As the target compounds can be simultaneously extracted, treated and analysed by our method, it can be hence used for routine analyses of pesticides, pharmaceuticals and mycotoxins in rice to provide large amounts of data. The analysis showed no occurrence of pharmaceuticals and mycotoxins in the rice matrices, but some pesticides were found in all the samples.

Keywords: *HPLC–MS/MS, insecticides, herbicides, antidepressants, antihypertensive drugs, mycotoxins*

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R-37
USE OF NEW SPHERICAL MATERIALS IN A DUAL-LAYER SPE TUBE FOR THE ANALYSIS OF PESTICIDE RESIDUES IN SPINACH

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Pesticide residue analysis of agricultural products has become commonplace in recent years. A popular approach to extraction of pesticides from fruit and vegetable samples involves the use of QuEChERS extraction followed by gas chromatography-mass spectrometry (GC–MS) or liquid chromatography-mass spectrometry (LC–MS) analysis. Highly pigmented samples such as spinach require cleanup prior to analysis for pesticide residues to avoid contaminating the analytical system. Cleaning these types of samples often involves the use of solid phase extraction (SPE) cartridges containing carbon in combination with aminopropylsilanized silica gel. Carbon has a strong affinity for planar molecules, and can remove pigments and sterols that are commonly present in many food and natural products. The aminopropyl (NH_2) sorbent functions to remove organic acids, and will also retain some polar pigments and sugars. Sample processing is normally done using gravity, to allow for sufficient contact time between the elution solvent mix and the SPE materials in the cartridge. Many dual-layer cartridges currently available are made using granular materials. These materials have variations in particle size and shape, which can lead to slower and more inconsistent flow between cartridges. In this work, we evaluated a new dual-layer carbon/NH_2 SPE cartridge containing spherical materials. Compared to SPE cartridges made with traditional granular materials, the new spherical materials were found to exhibit improved and more consistent flow characteristics. The new dual-layer SPE cartridge was evaluated with spinach extracts for color removal and pesticide recovery. The pesticides chosen for the study covered a variety of groups, and included polar and non-polar compounds. The new spherical materials were found to be highly effective at removing the chlorophyll present in the spinach extracts, and yielded better recoveries for a majority of the pesticides studied compared to granular materials.

Keywords: *Pesticides, QuEChERS, Spherical Materials, Carbon, Spinach*

R-38
RAPID DETECTION OF 250 PESTICIDE RESIDUES IN OKRA USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

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Okra is considered to be an important dietary constituent in the Indian subcontinent. According to the Food and Agriculture Organization of the United Nations (FAO), India is the second largest producer of this vegetable in the world and it generated approximately 5.8 million tonnes of okra in 2011. Okra is susceptible to a variety of pests and diseases and, therefore, a wide range of pesticides are used to treat okra plants in India. Legislative limits are in place for the presence of pesticides in domestically produced, imported, or exported okra. It is, therefore, very important to monitor commonly used pesticides at legislative limits. A multi-residue analysis method for the detection of 250 pesticides in okra will be presented. Samples were extracted using either acetate or citrate buffered QuEChERS methods. The extracts were subjected to a dispersive solid-phase extraction using different combinations of $MgSO_4$, PSA, GCB and C18 to determine the most appropriate method to further clean-up okra extracts prior to analysis. Ultra performance liquid chromatography coupled with tandem quadrupole mass spectrometry using 2 MRM transitions per compound were collected for all pesticides in either ESI+ or ESI- mode using rapid polarity switching. Simultaneous full scan data was acquired in order to assess any matrix effects. Product ion confirmation (PIC) scans were also acquired simultaneously to confirm pesticide identifications. For all pesticides, the limit of detection (LOD), the limit of quantification (LOQ) and recoveries at 10 µg/kg will be presented.

Keywords: *Okra, pesticides, LC-MS/MS, quadrupole, multi-analyte*

R-39
A SURVEY ON PESTICIDE RESIDUES OF IMPORTED FRUIT CIRCULATED IN GYEONGGI PROVINCE, SOUTH KOREA

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The various fruits are consumed for improving the standard of living in Korea and the consumption of imported fruits in open markets is rapidly increased. Thus, the concern of pesticide residues in fruits imported from developing countries is growing to keep the food safety. This study was performed to monitor the pesticide residues of imported fruit circulated at Gyeonggi province in Korea. Also, the separated experiment of peel and flesh was conducted to compare the current status of pesticide residues. The 124 imported fruits samples (22 different items) were tested for pesticide residues. The analysis of pesticide residues was conducted according to the Korea Food Code. Total 218 pesticides were analyzed by multi-residue method using gas chromatography/nitrogen phosphorus detector-electron capture detector(GC–NPD/ECD), time of flight/mass spectrometer (TOF/MS), ultra performance liquid chromatography/ photo diode array(UPLC–PDA), high performance liquid chromatography/fluorescence detector (HPLC–FLD) and mass spectrometer (LC–MS/MS). The pesticides were detected in 18 samples, ranging 0.003~0.3 mg/kg. Any samples did not over the maximum residue limits of pesticide residues established by Korean Food and Drug Administration (KFDA). In the separated experiment of peel and flesh, the pesticides were detected in 14 peels ranging 0.03~1.5 mg/kg. These results indicate that imported fruits are safe about pesticide residues but the potential risk of pesticide residues in imported fruits will be existed in Korea. Therefore, the pesticide residual amounts of imported fruits should be constantly monitored for the food safety.

Keywords: *Pesticide residues, peel, flesh, imported fruits*

R-40
METHOD DEVELOPMENT AND METHOD VALIDATION IN TEA AND INFUSION SAMPLES

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Tea is a commonly consumed beverage. Not only black tea and green tea are very popular, but also herbal tea products like rooibos or Paraguay tea are consumed frequently. As in other areas of agriculture, pesticides are used in tea cultivation and storage, which can lead to pesticide residues on the tea leaves. Because of the health risk they pose, these residues have to be controlled. The determination of numerous pesticides relevant in tea cultivation is performed by GC–MS or GC–MS/MS. Clean extracts should be applied to the GC–MS system to keep up a good performance. Sample preparation typically involves the extraction of the contained analytes with a suitable solvent and a clean-up step to remove further matrix constituents. With this approach well-cleaned measurement extracts should be obtained, which is desirable since undisturbed chromatograms facilitate evaluation. In the last few years the QuEChERS method [1] has been used frequently to analyse pesticide residues in plants as well as in tea. However, procedures which are derived from the QuEChERS method only include some of the usual steps of sample preparation. In the present study three different types of sample preparation procedures were tested for green tea:
a) The pesticides were extracted by a mixture of toluene and ethanol. The extract was cleaned by dSPE with PSA and graphitized carbon black, followed by gelpermeation chromatography.
b) The extraction of the pesticides was performed according to the QuEChERS method with acetonitril. A liquid-liquid-extraction was carried out as clean-up step [2].
c) The hexane extract of b) was purified by SPE using silica gel cartridges.
The results show that a laborious sample preparation not necessarily leads to a good analytical performance. According to Cajka et al. [2], a sufficiently clean extract for the determination by GC–MS/MS is obtained by submitting the QuEChERS raw extract to a liquid-liquid-extraction with hexane. In the current study, the hexane extracts of green, black, rooibos and Paraguay tea were subjected to silica gel in a further purification step to achieve a better matrix separation. The analytical results of the purified and unpurified hexane extracts were compared. For some analytes better performance indicators are achieved after the additional purification step, and the validation criteria of SANCO/12495/2011 [3] are met.

[1] Amtliche Untersuchungsverfahren nach §64 LFGB L00.00-115 (2007), Beuth Verlag ed. BVL
[2] T. Cajka et al., Analytica Chimica Acta 743 (2012) 51–60
[3] SANCO/12495/2011: Method Validation and Control Procedures for Pesticide Residues in Food and Feed

Keywords: *Tea, sample preparation, GC-MS, GC-MS/MS*

R-41
STUDY OF CO-EXTRACTED MATRIX COMPOUNDS AS INTERFERING COMPONENTS FOR THE ANALYSIS OF PESTICIDES IN FRUIT AND VEGETABLES

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The complexity of certain matrices can cause problems with the ionization efficiency of the analytical instruments. These problems in some cases lead to signal suppression effects and false negative occurrences. Furthermore, the presence of matrix compounds with very similar masses to target analytes could be a major drawback for an unequivocal identification and therefore false positive detections. The higher the complexity of the sample, the more false negatives and/or false positives will appear. The aim of this work is the study and chemical evaluation of co-extracted compounds as interfering components for the analysis of pesticides in relevant fruit and vegetables matrices. Four matrices (tomato, pepper, leek and orange) were extracted by the QuEChERS method and analysed by LC–TOFMS. Matrix compounds were retrieved and counted using the Molecular Feature Extractor (MFE) algorithm in the MassHunter workstation software. Co-extracted were compared between different matri ces and between different varieties of the same type matrix. The different matrices were spiked with a mix of pesticides and some matrix interference effects were evaluated. The results show that the number and distribution of interfering matrix components varies greatly depending on the particular vegetable matrix; even those included within the same commodity category according to EU guidelines [1]. The number of co-extracted matrix compounds with an absolute height ≥ 10,000 counts, is clearly higher in orange extract (8017 compounds), followed by leek (5870 compounds), pepper (3419 compounds) and tomato (2408 compounds). The number of isobaric compounds for a large multiresidue method (300 pesticides) is high in all studied matrices, but mainly in complex matrices such as leek and orange. In the mass difference range of 0 to 1 Da, more complex matrices (leek and orange) have over 9000 isobaric compounds and cleaner matrices (pepper and tomato), have over 3000 isobaric compounds. Signal suppression due to co-eluting matrix compounds would be partially solved through extract dilution. In leek the number of co-eluting matrix compounds decreases considerably with succeeding extract dilutions. In the 7–13 min range, with a dilution factor of 10, the number decreases to around 20% (2434) and with a dilution factor of 50, the number diminishes to 1410 interferences.

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Keywords: *Pesticides, fruit, vegetables, LC–TOFMS, interfering components*

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R-42
MONITORING OF PESTICIDES IN HOP AND CORRESPONDING HOP PRODUCTS USING AN IN-HOUSE DEVELOPED MULTI-RESIDUE METHOD

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A multi-residue method was developed for the determination of several pesticides (acetamiprid, alpha-cypermethrin, avermectin B1a, azoxystrobin, bifenthin, boscalid, captan, cinidon-ethyl, clothianidin, cymoxanil, cypermethrin, dimethomorph, fenpropimorph, fenpyroximate, flonicamid, fluazifop-p-butyl, fluopicolide, folpet, hexythiazox, imidacloprid, lambda-cyhalotrin, linuron, mandipropamid, metalaxyl, myclobutanil, propamocarb, propargite, pymetrozine, pyraclostrobin, pyraflufen-ethyl, quinoxyfen, spirodiclofen, tebuconazole, terbuthylazine, thiamethoxam, tolylfluanid, triadimenol, trifloxystrobin, zoxamide) in dried hops and hop products. Solid phase extraction (SPE) methods were used to clean up the extracts prior to GC–MS/MS and HPLC–MS/MS analysis. The recovery rates for all compounds were at least 70 %. The limits of quantitation (LOQs) in dried hop material ranged between 0.02 and 0.1 ppm, depending on the compound. LOQs of hop extracts were higher with factor 2–3. It was possible to detect 16 active ingredients (azoxystrobin, boscalid, dimethomorph, flonicamid, folpet, hexythiazox, imidacloprid, mandipropamid, metalaxyl, myclobutanil, pymetrozin, pyraclostrobin, quinoxyfen, spirodiclofen, triadimenol, trifloxystrobin) in different concentrations in samples analysed during the season 2012/2013. All results were always below the maximum residue levels (MRLs). To observe the behaviour of pesticides during hop processing, the analysis of dried hop samples (hop variety Perle, crop 2012) and the corresponding ethanol extracts as well as the results of the residue analysis of hop pellets (type 90, hop variety Hallertauer Magnum, crop 2012) and the corresponding carbon dioxide extracts were presented here. The investigations of the active ingredients described above showed that during the production of an ethanol extract the recovery rates of pesticides were higher than 70% with exception of pyraclostrobin (16%), folpet (50%), and trifloxystrobin (65%). The calculation of the expected amounts was done with the so-called enrichment factor which consists of the used amount of the raw material as well as the amount of the finished product. In comparison to ethanol extracts, the recovery rates of pyraclostrobin, folpet, and trifloxystrobin during the production of the carbon dioxide extract were about 80%, respectively. Most of the other pesticides determined in the CO_2 extract also showed recovery rates higher than 70%. Only the extraction amounts for boscalid, dimethomorph, and mandipropamid were lower, between 47–66%. The in-house developed multi-residue method enables the accurate determination of pesticides not only in basic raw material like dried hop but also in all hop products like hop extracts or pellets.

Keywords: *Dried hop, hop products, multi-residue method, pesticides*

R-43
RAPID AND SIMPLE APPROACHES TO MULTI-RESIDUE PESTICIDE ANALYSIS IN FRUITS AND VEGETABLES ON BOTH GC–MS/MS AND LC–MS/MS

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Tandem mass spectrometry coupled to chromatography, such as GC–MS/MS and LC–MS/MS, operated in MRM mode, has become the method of choice for targeted screening of multi-residue analysis in complex food matrix samples. A fast, easy and efficient sample preparation of food sample is the key to multi-residue pesticide MS analysis, which in fact still remains as a challenge. On the other hand, the multi-residue MRM method is labor-intensive and time-consuming. For production labs, it is always desired to have a ease-of-use software which integrates MRM method development flow to significantly speed up the method set-up. In the current study, an improved QuEChERS sample preparation protocol as an alternative to the conventional QuEChERS is employed for vegetable matrix extraction. It is easy, fast, and has comparable recovery rate. Three vegetables rice, spinach and avocado representing low moisture, high moisture, fatty sample respectively, were applied with the improved QuChERS method for extraction. A pesticide mix containing 30 residues was spiked into each extracted QuEChERS matrix for calibration and analysis. The exacted matrix is in acetone solvent, therefore can be diluted and directly shoot into GC–MS and LC–MS for pesticide analysis, which largely simplifies sample prep and saves time. Good sensitivity of low ppb (1 ppb) on GC–MS/MS and sub-ppb (0.1 ppb) on LC–MS/MS were demonstrated. Great linearity was achieved on both instruments as well. We also demonstrate MSWS 8.1 software with Compound Based Screening (CBS) workflow for fast MRM method development using Bruker Scion GC–MS/M S and EVOQ LC–MS/MS system. This study provides a complete solution for pesticide analysis in vegetable samples from sample prep to MS analysis.

Keywords: *Improved QuEChERS, vegetable, GC–MS/MS, LC–MS/MS*

R-44
EVALUATION OF MATRIX EFFECTS ON DILUTE–AND–SHOOT LC–MS/MS ANALYSIS OF CARBENDAZIM IN ORANGE JUICE AND WINE

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Carbendazim, a banned fungicide in the US was detected in orange juices imported from Brazil (2011) and in Chinese brand wines (2012), which has triggered the high demands for carbendazim screening in juice produces. LC–MS/MS is a powerful technology for trace level pesticide analysis in food. Sample preparation of pesticide analysis in juice generally employs two strategies: solid phase extraction (SPE) or dilute-and-shoot. Dilute-and-shoot is preferred due to its simplicity; however no comprehensive study has yet been done to evaluate dilution-factor-caused matrix interference for carbendazim analysis. In the current study, an quantitative MRM method for carbendazim analysis in orange juice and wine matrices was developed using a Bruker EVOQ™ LC–MS/MS system with a calibration range from 0.005 up to 50 ppb (ng/mL). Good instrument sensitivity (LOQ 0.005 ppb) and robustness were achieved during method development. Matrix effect was evaluated by comparing matrix effect% against during dilution factors. Our results show that orange juice and wine matrices exhibit different matrix effects and provide the scientific reference to determine the appropriate dilution factor for carbendazim analysis in juice and wine. Finally, the diluted orange juice samples and wine samples were tested using the currently established method for carbendazim screening.

Keywords: *Carbendazim, matrix effect, orange juice, wine*

R-45
QUECHERS SAMPLE PREP AND ITS CLEANUP EFFECTIVENESS AND EFFICIENCY – A LOOK AT SORBENTS

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Pesticide analysis In both food and wastewater typically necessitates sample preparation. Matrices including fruits and vegetables, meats, botanicals and coffee, and wastewater all necessitate cleanup before analysis with GC–MS or LC–MS/MS. QuEChERS (quick, easy, cheap, effective, rugged, and safe) cleanup can be used for all of these matrices. Cleanup usually involves combinations of primary secondary amine (PSA) to remove fatty acids, C18 functionalized silica to remove non-polar contaminants, and graphitized carbon black (GCB) to remove pigments and sterols. This last sorbent, GCB, introduces issues with the recovery of planar pesticides. This poster discusses alternatives in the form of engineered carbon adsorbents that minimize or eliminate this issue, while retaining pigment sorption during a QuEChERS style cleanup of multiple matrix types. These sorbents (CarbonX Plus and the new CarbonX BoS) both have fully porous metal oxide substrates covered in carbon. CarbonX Plus utilizes pyrolytic carbon while CarbonX BoS utilizes a C60 Fullerene bonded phase. Both allow for improved recoveries of planar pesticides when compared to GCB in a QuEChERS cleanup method.

Keywords: *Pesticides, Carbon, Sample Preparation, Porous*

R-46
THE QUECHERS THREE MINUTES EXPERIENCE – SCREENING & TARGETED DATA ANALYSIS ON THE FLY

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In last decade, there has been continuously growing public concern in contamination of food with anthropogenic compounds and their degradation products and the evident negative influence to the public health. As a response to this fact, there is an increased interest in more efficient screening techniques of larger number of possible pollutants compared to those are carried out by triple quadrupole mass spectrometers. The high resolution and accurate mass spectrometry approach does not require optimization of compound specific parameters and has the ability to properly separate matrix from compounds of interest. In this work, routine QuEchERS samples have been tested with new scanning techniques by using a second generation Exactive OrbitrapTM system. The experiment consisted of full scan mode (resolving power 70,000 FWHM) with “data dependant All Ion Fragmentation mode” (ddAIF) acquired at a resolving power of 17.500 FWHM for spectral library confirmation of known and unknown screening. The fragmentation spectra were generated by using a high energy collision induced dissociation cell (HCD). These experiments were tested and evaluated in terms of quantification and confirmatory capabilities for the analysis of a standard mixture of 150 pesticides in positive and negative ionization mode within two 3 minutes chromatographic runs. The used approach led to reduction of matrix interferences and gave higher sensitivity for quantitation plus more precise data dependant spectra for confirmation side by side also without mass selection using a quadrupole. In combination with a new workflow covering software suite it was possible to acquire & process data on the fly to speed up both data review and reporting.

Keywords: *HRAM, U-HPLC, Pesticides, Residues*

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R-47
RESIDUES OF PESTICIDES AND CONTAMINANTS IN AGRICULTURAL CROPS MONITORED BY BRAZILIAN MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY

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The National Plan for Control of Residues and Contaminants in Plant Products (PNCRC/Vegetal) began in 2006, driven by requests in relation to quality assurance and hygienic-sanitary safety of products and by-products from plant origin. Normative Instruction No. 42 of December 31th, 2008, represents the legal framework for the PNCRC/Vegetal, which has as main purpose to monitor plant products and the plant production systems in order to identify hazards, evidence-based analytics findings, and to interfere in order to eliminate or to minimize the chemical risks through regulation, guidance and fomentation. The PNCRC/Vegetal oversees and inspects many granted and registered inputs, forbidden and unregistered pesticides as well organic and biological contaminants in Brazil. The official samples are collected on farms, processors establishments, supermarkets and supply centers by Federal Inspectors from Ministry of Agriculture, Livestock and Food Supply (MAPA), a nd each sample is identified by an individual number which ensures full traceability. The official sampling follows the recommendation from Codex Alimentarius. The analyzes are carried out by the MAPA's Official Laboratories Network, which is constituted by official laboratories, the National Agricultural Laboratories (LANAGROs), and by some public and private permited laboratories. All of the labs must be accredited in ISO/IEC 17025 by the National Metrology, Quality and Technology Institute (INMETRO). The PNCRC/Vegetal started off monitoring Apple and Papaya and these production systems, and from 2011–2012 crop year on it includes 22 crops: Pineapple, Lettuce, Peanuts, Rice, Banana, Potato, Coffee, Brazilian Nuts, Citrus, Beans, Apple, Papaya, Mango, Melon, Corn, Strawberry, Black Pepper, Pepper, Soybean, Tomato, Wheat and Grape, representing an increase about of 1000%. At first, these crops were monitored for only 86 different types of residues of pesticide and contamin ants, and then the scope widely increased to 241 monitored substances. From 2006 on 3.937 official samples were analyzed and the most of non-compliant results (analytical results above the established MRL or detection of non-permited substances for a specific crop) occurred in Lettuce, Brazil Nuts, Mango, Strawberry, Peach, Pepper, Soybean and Wheat. These non-conformities were identified as inappropriate use of approved substances, inadequate storage conditions of crops, deviation of use of registered inputs and some as the use of forbidden substances. This results assessment infers a situational diagnosis about the residues and contaminants in agricultural crops in Brazil. It is necessary a coordinated and collective work of the official controlers, producers, farmers and enterprises related to plant products, in order to mitigate new non-conformities, improving the quality and safety of the plant products consumed in Brazil and exported to trading partners.

Keywords: *Pesticides, Residues, Plant Products*

Acknowledgement: *Ministry of Agriculture of Brazil*

R-48
EVALUATION OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES FROM THE REGION OF SOUTH-EASTERN POLAND

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The aim of this study was to determine the presence of pesticide residues in Polish fruits and vegetables and to assess if these residues pose a risk to the health of the consumer. Furthermore, compliance with legal regulations concerning the use of plant protection products in crop cultivation was ascertained. In 2010–2012, 1026 unprocessed samples of fruits and vegetables from south-eastern Poland were tested in the laboratory. The tests covered the determination of pesticides, from 138 in 2010 to 167 in 2012. Methods accredited by the Polish Centre for Accreditation were used to determine the presence of pesticide residues (ISO/IEC 17025:2005). Test results were confirmed in accordance with European Commission guidelines (SANCO 2011 Document). The obtained results were compared with the Maximum Residue Limits in force in both Poland and the European Union. It was additionally verified whether the pesticide was recommended for use in a given crop. Pesticide residues were found in 376 samples (36.6% of all tested samples): in 277 (50.6%) samples of fruits and in 99 (20.7%) samples of vegetables. Among fruit samples, the most pesticide residues were found in: gooseberry (66.7% of samples), apple (61.9%), grape (50%) and black currant (48.6%) while among vegetable samples: celeriac (62.5%), tomato (59.5%), sweet pepper (55.6%) and Peking cabbage (53.8%). 43 active substances were found: 24 of them were fungicides, 15 were insecticides, and 4 were herbicides. The following fungicides were found the most often: captan (12.4% of analysed samples), dithiocarbamates (5.6%), and pyrimethanil (4.6%) while the most often found insecticides were: chlorpyrifos (5.2%), cypermethrin (3.1%) and pirimicarb (1.9%). In 18 samples (1.8%), residues exceeded Maximum Residue Limits: in 12 (2.2%) samples of fruits and in 6 (1.3%) samples of vegetables. Analyses of samples also showed the presence of banned pesticides. There were 13 cases where such substances were found, i.e.: diazinon, fenarimol, fenitrothion, procymidone, tolylfluanid and trifluralin. The banned substances were found in apple (9 samples), carrot (2), cherry (1) and cucumber (1). In 28 (2.7%) samples, substances not recommended for a given crop were detected. Almost half of samples with residues contained multiple residues. Multiple residues were found most frequently in: apple, black currant, tomato, Peking cabbage, cherry and strawberry. The highest values of long-term exposure were for dimethoate residue in apple (1.7% ADI, adults; 6.8% ADI, toddlers). For most detected pesticides, long-term exposures were below the values of 1% ADI for adult and 3% ADI for toddlers. The highest values of short-term exposure were obtained in the case of consumption of apples with azoxystrobin (4.5% ARfD, adults; 13.3% ARfD, toddlers).

Keywords: *Pesticide residue, fruit, vegetable, dietary exposure, intake*

R-49
AUTOMATED SAMPLE PREPARATION AND ANALYSIS WORKFLOWS FOR PESTICIDE RESIDUE SCREENINGS IN FOOD SAMPLES USING DPX–QUECHERS WITH LC–MS/MS

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One of the most important aspects of reducing pesticide exposure is to monitor for pesticide residues in foods due to the health risks they pose to humans and livestock. A number of analytical methods have been published, many of them based on traditional liquid-liquid extraction in combination with GC–MS or LC–MS. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods have been developed to help monitor pesticides in a range of food samples. These methods, however, still require many manual steps, such as shaking, mixing, centrifugation, and dispersive SPE, making it a quite labor-intensive process. There is a need for automating the dispersive SPE technique to clean up QuEChERS type extracts in order to improve laboratory productivity for monitoring pesticide residue in complex food matrices. In this report, we describe an automated sample preparation and analysis workflow for the screening of pesticides residues in different food matrix (fruits, vegetables and nuts) by LC–MS/MS. The automated cleanup of the QuEChERS extracts methodology was performed using disposable pipette extraction (DPX). DPX is a solid-phase extraction (SPE) technique that is based on loosely contained cleanup sorbent inside a pipette tip fitted with a screen. Analytical methodology for confirming the presence of a variety of pesticides in various food samples was developed using a GERSTEL MPS robotic autosampler interfaced to an AB SCIEX QTRAP® 4500 LC/MS/MS System. Two transitions per parent compound were monitored using a single a Scheduled MRM™ method. The automated DPX-QuEChERS cleanup procedure provided extraction efficiencies greater than 70% for all pesticides screened in the different food samples with RSDs less than 15%. In addition; good linearity was achieved (R^2 values of 0. 98 or greater) allowing detection limits of the method to meet acceptance criteria for reporting maximum residue levels (MRLs) as established by regulatory agencies. The ability to automate dispersive SPE clean-up of QuEChERS extracts and to couple the extraction directly to LC–MS/MS analytical methods, results in improved laboratory productivity by streamlining the complete analytical process.

Keywords: *QuEChERS, Automation, LC–MS/MS, Sample Preparation, Pesticide Residues*

R-50
GOT MILK? GET IT CHECKED: ORGANOCHLORINE AND PCB RESIDUES IN SELECTED CZECH DAIRY PRODUCTS

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Dairy products form an indispensable component of human diet in many countries. The range of dairy products consumed in Czech Republic ranges from fresh milk with varying fat content, cream products such as “smetana” (Czech sour cream), sour milk products such as “tvaroh” (Czech curd cheese), yogurt, butter and “Olomoucké tvarůžky”, an aged cheese with a strong odour unique to the Czech dairy section. As dairy products are an excellent source of both macro- and micro-nutrients, both children and adults consume them in one form or another. However, mammalian milk tends to accumulate pesticide residues. Such pesticides have been reported to have a slew of detrimental effects to human health. Though such pesticides have been banned for many years now, they can still be detected due to its persistent nature. Methods of screening such persistent pesticide residues have largely employed liquid-liquid extraction methods that use large quantities of h azardous solvents and require hours of preparatory steps. Recent methods employ a solid-phase dispersive method which uses a fraction of the solvents, saves time and provide reliable results, QuEChERS being probably the most outstanding approach. There has been no comprehensive comparison of pesticide residues in Czech dairy products using the QuEChERS method. Thus, the objectives of this work are two-fold:
1) screening of Czech dairy products for organochlorines and polychlorinated biphenyls (PCBs) in cow’s milk from different sources, “smetana”, “tvaroh”, yogurt, butter and “Olomoucké tvarůžky”,
2) the effectiveness of the QuEChERS method in recovering these pesticides from dairy products with varying fat-content. The analytes were quantified using a Gas Chromatograph equipped with an Electron Capture Detector (GC–ECD). The presentation will address in detail the results of the screening and will discuss differences in pesticide content among the tested da iry products.

Keywords: *Dairy products, GC–ECD, organochlorine residues, PCB residues, QuEChERS*

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R-51
THE COMBINATION OF QUECHERS AND DLLME FOR THE DETERMINATION OF DIFFERENT CLASSES' PESTICIDE RESIDUES BY GC–MS (ECD) IN FOOD

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Combined pesticides (mixture of two or more active substances of pesticides) are widely used nowadays. This approach is an important reserve for increase the biological and economic efficiency of the application of chemical products for plant protection. Typically, the active substances of combined pesticides belong to different classes. Furthermore, throughout the "lifetime" to the consumer product is treated by a lot of different classes' pesticides. Therefore, the question of the simultaneous determination of various classes' pesticide residues remains important. In the Russian Federation, the issue is particularly acute because there are no methods of simultaneous determination of the active substances of different classes' pesticides in products of plant and animal origin. Few techniques aimed for identifying small number of different classes' pesticides (2-3) in the various products. In the present work we showed the possibility of simultaneous determining of 25 organochlorine pesticides (OCP's), 22 organophosphorus pesticides (OPP's), 10 pyrethroids, 11 triazole derivatives and captan by gas chromatography-mass spectrometry (GC–MS) with electron ionization in positive ion mode and 15 organochlorine pesticides, 11 organophosphorus pesticides 5 pyrethroids, 4 triazole derivatives and captan by gas chromatography with electron capture detection (GC–ECD) in vegetables, fruits, grains, milk, meat and honey. The target components were extracted by QuEChERS and extract was concentrated by dispersive liquid-liquid microextraction (DLLME). The proposed method reduced the limit of quantification (LOQ) to 0.08–2 μg/kg. Relative standard deviation is less than 0.09. Calibration characteristics are linear in the range of 0.1–2 μg/ml (R^2 ≥ 0,996). The recoveries were between 50 and 100% depending on the matrix. Duration of analysis is about 1 hour. DLLME method allowed not only to concentrate the sample by 40 times, but also to clean up extract considerably and increase the recoveries.

Keywords: *Pesticides residues, QuEChERS, DLLME, GC-MS, GC-ECD*

R-52
PARAMETERS AFFECTING THE PERFORMANCE OF LC–HRMS SCREENING METHODS FOR MULTICLASS SCREENING OF 600 ORGANIC CONTAMINANTS IN FOOD BASED ON ACCURATE-MASS DATABASE

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The presence of organic contaminants in food represents a serious health issue. The use of liquid chromatography-high resolution mass spectrometry-based screening methods makes possible to analyze hundreds of contaminants in a single run. However, this is a challenging task difficult to tackle with. Leaving aside the sample preparation step, which must be as universal as possible in this type of methods –although in real practice it is impossible to find such method-, there are different parameters affecting the performance of LC–HRMS screening methods. Amongst them: chromatographic, data acquisition and data processing parameters. Mass spectrometric acquisition and chromatographic parameters of the screening methods play an important role on the method performance, including both sensitivity and selectivity (limits of detection, matrix effects, etc.). In addition, data processing parameters such as automated searching parameters (eg. *m/z* error tolerances, retention time windows, minimum intensity values, …) using specific LC–HRMS software are also important to avoid the chance of false negatives/false positives. In these methodologies there are also interference issues because of the huge number of coeluting compounds included that can be detected in a single run. The aim of this work is to evaluate different chromatographic, data acquisition and data processing parameters that affect to screening methods. This study, based on a screening method developed for 600 multiclass contaminants, has been performed using Ultra High Pressure Liquid Chromatography High Resolution Mass Spectrometry with a time of flight mass spectrometer (Agilent 6220). The database comprises retention time and accurate-mass values for each compound. An Agilent UHPLC system (Agilent 1290 Infinity) was used with a high definition reversed phase C18 analytical column of 50 mm × 2.1 mm and 1.8 μm particle size (Zorbax Rapid Resolution High Definition (RRHD) Eclipse-Plus C18). Mobile phases A and B were water and acetonitrile both with 0.1% formic acid,with a flow-rate of 0.5 mL min-1. Different retention time windows (±0.05, ±0.1, ±0.25, ±0.5 min) and *m/z* tolerance windows (5 and 10 ppm) of the automatic search tool were tested. In addition, 20 representative compounds were selected to optimize chromatographic and acquisition parameters. The chromatography was evaluated using different columns with different sizes, operating at different pressures. Then, various elution gradients were assayed (5, 10 and 15 minutes). Different injected sample volumes (2, 5, 10 and 20 μL) were also evaluated to study the influence on both matrix effects and sensitivity. Mass spectrum acquisition rate was also considered (from 0.5 to 10 Hz). Finally, several compounds with similar retention times were selected to evaluate potential coelution problems in this type of large-scale screening methods and the problems associated during validation step.

Keywords: *Accurate-mass database, organic contaminants, food, screening, liquid chromatography-mass spectrometry*

R-53
A STRATEGY FOR AN AUTOMATED UNKNOWN SCREENING APPROACH ON ENVIRONMENTAL SAMPLES USING HRAM MASS SPECTROMETRY

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Introduction. The analysis of food and environmental samples for contaminants by LC-MS has become a quick and cost effective routine application when run in a targeted fashion, but this way it disregards events or circumstances not taken into account beforehand. Run in a non-targeted fashion, it is known to be laborious and time consuming, making it everything but a routine application. New generation software now links in quantitative and (unknown) screening approaches to one smoothly integrated workflow, tying together component detection capabilities of unknown screening workflows with the identification capabilities of targeted screening and quantification software. Here we show how one data set can serve for routine high throughput quantitative analysis and for versatile non-targeted investigations in a highly automated manner.
Methods. Surface water samples were analyzed on a Thermo Scientific Exactive Plus™ mass spectrometer coupled to a Thermo Scientific EQuan™ chromatography system serving for online concentration and analysis in one step. 1 mL of each sample was injected onto the system without any pretreatment, using a 6.7 min gradient of water and acetonitrile (each with 0.1% formic acid), resulting in a 15 minute chromatographic cycle including online sample concentration. The mass spectrometer was run in full scan mode alternated with all ion fragmentation (AIF) scanning, using a resolution of 35,000 @ *m/z* 200.
Preliminary data. Different surface water samples were analyzed in order to determine contaminants in an non-targeted approach. After data acquisition, processing was triggered for compound detection and differential analysis. The processing software normalizes the results to a reference sample, for which a drinking water sample from outside Berlin was used. Subsequently all results were filtered for significant differences in relation to the reference by choosing a difference factor of greater than 10. Components passing this criterion were passed on to an automated internet search using ChemSpider™. The search result was passed back automatically into the LC–MS application software for confirmation. This was achieved by using the ChemSpider search result for a targeted screening on the raw data, using retention time, isotopic pattern match and S/N ratio as quality criteria. Multiple compounds from various groups such as pesticides, pharmaceuticals, drugs and personal care products could be identified. Novel aspect Automated workflow from sample preparation over data acquisition to compound identification for general unknown screening in one software solution

Keywords: *General Unknown Screening, HRAM, Orbitrap, online solid phase extraction*

R-54
IMPROVING CONFIDENCE IN THE IDENTIFICATION OF PESTICIDE RESIDUES BY GC–MS USING TARGET DECONVOLUTION

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Unit mass resolution single quadrupole gas chromatograph – mass spectrometer (GC–MS) systems have been in routine use for both qualitative and quantitative analyses for the past 25 years. When employed for quantitative analyses, target analyte identification is typically performed by using retention time and the ratio of up to 3 qualifier ions to a single quantitation ion. Whilst this identification method works well in relatively 'clean' chromatograms, more complex chromatograms such as those resulting from the extraction of food or environmental samples can contain co-extracted matrix components. These interferent components can cause both false positive and false negative results, leading to more time spent on data review. Increased selectivity against matrix can be achieved by using mass spectral deconvolution and comparison of the mass spectra of deconvoluted components to a reference database of mass spectra. This poster details a new feature of Agilent's Mass Hunter Quant software (Ver. B.06.00) called Target Deconvolution (TD). TD provides fully integrated mass spectral library matching of deconvoluted component spectra alongside the use of retention time and ion ratios to provide increased confidence in the identification of target analytes by full scan GC–MS analysis. Fast data processing and the powerful graphical features of Batch-at-a-glance and Compounds-at-a-glance provide fast and efficient data review, especially when used in combination with Mass Hunter Quant outlier flagging.

Keywords: *Pesticides, Residues, GC–MS, Deconvolution*

R-55
RAPID AND SIMULTANEOUS IDENTIFICATION AND QUANTITATION OF PESTICIDES IN DIFFERENT MATRICES USING HIGH SENSITIVE LC–MS/MS

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LC–MS/MS instruments operating in Multiple Reaction Monitoring (MRM) are widely used for targeted quantitation on triple quadrupole and hybrid triple quadrupole linear ion trap (QTRAP®) systems because of their well known selectivity and sensitivity. Recent regulations on food and environmental analysis require screening for pesticides using confirmatory techniques, such as GC–MS and LC–MS/MS. More than 1000 pesticides are used worldwide and, along with their metabolites and degradation products, are present in food and the environment. Thus, there is demand for powerful and rapid analytical methods that can detect very low concentrations of pesticides. The big challenges for pesticide residue laboratories at the moment are the requests to test for larger number of compounds, in a wider range of commodities, in shorter time, all without sacrificing data quality. We will present the possibility to analyze multiple pesticides, for example, Dinocap, Iprodione, Fenavalerat, Cyalothrin, at a required detection level by EU regulation in different matrices such as parsley. The implemented LC/MS/MS screening method can be also simultaneously used for quantitation. AB SCIEX QTRAP® 6500 system with IonDrive™ technology enabling unprecedented sensitivity together with an ultra-fast HPLC System, provide the capability to analyze difficult matrices previously extracted by the QuEChERS multiresidue method, described in EN 15662, and subsequently diluting "quantum satis" in order to avoid the matrix effects that affect the response of target analytes in sample extracts compared to the response of standard solutions in pure solvent. Scheduled MRM with fast pos/neg switching was applied here for measurement. SelexION™ Technology which is compatible with QTRAP® 6500 systems shown combined sensitivity and selectivity gains. Detection limits of various pesticides were found to meet the required values by EU standards. With this study we can demonstrate the possibility to analyze difficult pesticides at the defined matrices avoiding the matrix effects that affect the response of target analytes in sample extracts and later on implement this into a robust LC/MS/MS screening /quantitation method. We demonstrated by using the new AB SCIEX QTRAP® 6500 system with ultra high sensitivity that both reproducibility and quantitation accuracy can be improved with no compromising on instrument sensitivity. The use of the sMRM acquisition technique with positive/negative switching has enabled shorter run times without the loss in sensitivity. Additionally a SelexION method for the analysis of acrylamid with increased LOQ and selectivity will be shown.

Keywords: *Pesticides screening, low level quantitation, Pos/Neg switching, EU Regulation*

R-56
PESTICIDE ANALYSIS AT OFFICIAL CONTROL OF ORGANIC AGRICULTURE IN THE CZECH REPUBLIC

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Central Institute for Supervising and Testing in Agriculture (UKZUZ) is responsible for official controls in various fields of agriculture production in accordance with the Law No 147/2002 Coll. In 2010 Ministry of Agriculture designated UKZUZ as an official body for performing controls in organic farming. In 2011 UKZUZ launched a programme for official controls of organic viticulture. A list of pesticides authorized for application on conventional vineyards in the Czech Republic includes 43 active substances. 30 pesticides (predominantly fungicides) were selected for control purposes. An accredited method based on the QuEChERS sample preparation and gas chromatography and/or ultra performance liquid chromatography coupled to tandem mass spectrometric determination was used for analyses. More than 130 samples of vine leaves and grapes were analysed in 2011–2013. Generally, the pesticides found in the inspected organic samples corresponded to those, which are commonly applied in the conventional viticulture. Folpet was the most frequently found and abundant pesticide. The levels usually pointed out unintentional pesticide introduction to the organic vineyards by airborne transport. Cases of fungicide misuse were rare and they are discussed in detail in the poster. In 2012 UKZUZ extended the scope of official controls to organic feed production area. The pesticide scope was similar to that monitored in conventional feeds. Unlike conventional samples, no pesticides were found in organic feed materials at levels above the reporting limits. Growth regulators were additionally analysed in the inspected samples. Even though these plant protection active substances are used in huge quantities at cereal and oil plant production the results have not indicated any incorrect practices related to this kind of organic samples.

Keywords: *Pesticides, organic viticulture, organic feed*

R-57
THE EFFECT OF THERMAL PROCESSING ON THE LEVEL OF PESTICIDE RESIDUES IN APPLES INTENDED FOR THE PRODUCTION OF FOODS FOR INFANTS AND YOUNG CHILDREN

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European Commission Directive No 2006/125/EC specifies acceptable levels of pesticide residues in processed cereal-based foods and baby foods for infants and young children as 0.01 mg/kg. Exceptions include substances for which the highest acceptable levels of residues in products intended for such applications are specified to be 0.004–0.008 mg/kg and prohibited substances. There are some substances for which maximum residue limits are set at the level of 0.004–0.008 mg kg^{-1} and some which are prohibited from use. Raw materials are very often rejected by companies that manufacture foods for infants and young children due to the presence of pesticide residues at a level significantly exceeding 0.01 mg/kg. In agricultural practice, the use of agents in accordance with the information on their labels, i.e., at correct doses and with observance of the prescribed waiting periods, does not guarantee that the final products will meet these rigorous parameters. Experiments were carried out to determine changes in pesticide residues caused by thermal processing. The purpose of these experiments was to assess the stability of some pesticides (fluquinconazol, spirodiclofen, difenoconazole, captan and pyrimethanil) in apples. Treatments were carried out at an orchard in Rzeszów (Poland) that supplies apples for the production of baby foods for infants and young children. Sampling was carried out after the waiting period expired, as provided for in the regulation on food sampling for determination of pesticide residue levels (Commission Directive 2002/63/EC). It was found that during roasting, the concentrations of tested pesticides: fluquinconazol, spirodiclofen, difenoconazole and pyrimethanil, increased, which was caused by the evaporation of water. An exception was captan, the residues of which significantly decreased.

Keywords: *Baby food, pesticide, residues, thermal, processing*

R-58
A RAPID SPECTROPHOTOMETRIC ASSESSMENT OF SELECTED ORGANOPHOSPHORUS PESTICIDE SCUMS IN VEGETABLE

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A new and highly sensitive spectrophotometric method was developed for the determination of parts per million levels of widely used organophosphorus insecticides, i.e. malathion, dimethoate and phorate is described. It is based on the oxidation of organophosphorus pesticide with slight excess of N-bromosuccinimide (NBS) and the unconsumed NBS is determined with rhodamine B (lambda max: 550 nm). Beer's law is obeyed in the concentration range 0.108–1.08, 0.056–0.56 and 0.028–0.28 μg mL^{-1} for malathion, phorate and dimethoate, respectively. The method has been successfully applied for the determination of organophosphorus pesticide residues in various vegetable samples. The method is simple, sensitive and free from interferences of other pesticides and diverse ions. Other pyrethroid insecticides do not interfere in the proposed method. The method has been satisfactorily applied to the determination of cypermethrin in environmental and biological sampl es.

Keywords: *Spectrophotometric, Sensitive, Concentration range, Organophosphorus pesticide, NBS*

R-59
COMPARISON OF RAPID AND CONVENTIONAL EXTRACTION METHODS FOR DETECTION OF 2DCB IN BRAZILIAN IRRADIATED FOODSTUFFS

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Irradiation of foods and foodstuffs in general has been considered as an important physical treatment in food technology. Both gamma and e-beam application are discussed now to be a good way to treat different kind of foods. Food irradiation is a process that consists of submitting the food already packed or in bulk at a carefully controlled amount of ionizing radiation for a predetermined time, with well-defined goals. Current Brazilian legislation as per Resolution – RDC No. 21 of January 26, 2001, says that any food can be treated with ionizing radiation whereas the minimum absorbed dose should be sufficient to achieve the intended purpose, and the maximum dose should be less than would compromise the functional properties and / or sensory attributes of the food. For each objective it is necessary to study the better condition and application to demonstrate and clarify to everyone that food irradiation process is used to improve the quality and give a saf e performance of the final product. Much has been discussed about the formation of dangerous chemical compounds formed by irradiation in foods containing fat. In order to provide more reliable information to consumer's confidence and present an economic, rapid and reliable method, we propose using two different radiation doses for detection of 2DCB in different types of Brazilian foods and compare the conventional extraction method as well as the European standard method EN 1785 (2003) for detection of 2-dcb with a rapid extraction method adapted using acetonitrile, in Brazilian irradiated foods.

Keywords: *Food Irradiation, Irradiation Detection, 2-DCB, Extraction Method*

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R-60
DOES THE QUECHERS SOLVENT ACETONITRILE DAMAGE GC COLUMNS?

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Claims of GC column damage from acetonitrile used for QuEChERS extractions have been reported often in presentations and scientific literature, but no definitive experimental results prove this to be true. We performed a ruggedness study on a 5% phenyl-type GC column, the stationary phase most used for multi-residue pesticide GC analyses, to define any performance degradation for pesticide retention time stability, pesticide response factor, and GC column stationary phase bleed that might result from using either acetonitrile or acetonitrile with 1% acetic acid, the two QuEChERS solvents. Repeated injections, over 1600, of 1% acetic acid in acetonitrile or 10% acetic acid in acetonitrile did not cause increased stationary phase bleed on the GC column. Organophosphorus pesticide retention times were extremely stable when the column was repeatedly temperature programmed to its maximum isothermal temperature of 330°C and its maximum programmable temperature of 350°C, showing less than 0.2% RSDs. Additional experiments where the column was continually programmed to 360, 370, 380, 390, 400, 410, and 420°C still yielded hardly any change in retention times, indicating surprisingly little loss of GC stationary phase under extreme operational conditions. Pesticide response factors and peak shapes, too, were very stable for the life of the experiment (including temperature programming to 420°C), except for 3 problematic pesticides, monocrotophos, azinphos methyl, and coumaphos, whose response factor variation was attributed to possible GC inlet liner deactivatio n degradation during solvent/standard injection. Ultimately, the protective polyimide polymer on the outside of the fused silica GC column was destroyed due to the very high temperature work, even before the stationary phase polymer on the inside, demonstrating the ruggedness of the 5% phenyl polymer and dispelling the myth of stationary phase damage caused by acetonitrile.

Keywords: *QuEChERS, acetonitrile, GC column, pesticides*

R-61
USE OF GC–QTOFMS WITH SOFT IONIZATION TO IDENTIFY PESTICIDE RESIDUES AND EMERGING CONTAMINANTS IN FOODS

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This paper describes the use of a GC–QTOFMS and a prototype soft ionization source to identify pesticide residues and a few emerging contaminants (i.e. polybrominated diphenyl ethers, phosphate esters, steroids, fatty acid esters) in selected certified standard reference materials and food matrices. The soft ionization source incorporates a microplasma discharge, as a source of vacuum ultraviolet (VUV) photons, and can be tuned by choice of plasma gas to operate in the range of 104–150 nm, corresponding to photon energies between 8 and 12 eV (i.e., Ar at 11.83 and 11.62 eV, Kr at 10.64 and 10.03 eV, Xe at 9.57 and 8.44 eV). In addition, this source utilizes a windowless design that allows operation with the full range of rare gas mixtures including those with emission lines below the ~105–115 nm cutoff of the VUV transmissible materials such as LiF and MgF_2. In addition to the "soft" ionization mode, the ionization source described here can also be op erated in a selective mode, where the photoionization energy is chosen to be less than the vertical energy of the solvent used for analysis or the components of the sample matrix. The combination of soft ionization and resolution >13,000 (at *m/z* 271.9867) will be illustrated with specific examples.

Keywords: *GC–QTOFMS, soft ionization, pesticides, emerging contaminants*

R-62
ACETYLCHOLINESTERASE BASED ELECTROCHEMICAL BIOSENSOR FOR CHLORPHENVINPHOS DETECTION; AN EFFICIENT SCREENING TOOL FOR SURFACE WATER ANALYSIS

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This communication presents the fabrication and evaluation in surface water samples of a low cost screen-printed biosensor for the detection of the pesticide Chlorphenvinphos (CFV), based on the inhibition of acetylcholinesterase from Drosophila melanogaster. CFV is listed as one of the priority substances for monitoring in surface water bodies [1]. The performance of developed screen-printed biosensor in real samples, analysed as such and after spiking with known amount of pesticides, has been investigated in relation to the maximum legal concentration for individual pesticides in drinking water in Europe. Relevant pesticides of different nature have been tested individually and in combination with CFV. A slight inhibition was observed in the cases where CFV was present (4%), as opposed to no inhibition when the rest of the pesticides were evaluated separately at the regulatory limit for drinking water (0.1 µg/L [2]) (Ethoprophos, Deltametrin, Terbuthylazi ne and Glyphosate). Additionally, mixtures of these pesticides with CFV did not vary in a large extent the response observed by the samples containing only CFV. The same way, addition of arsenic in the regulatory level to the CFV containing sample, did not interfere in the results. The limit of detection of the biosensor towards Chlorphenvinphos in real surface water was 6.2×10^{-10} M which corresponds to 0.22 µg/L. Stability studies of the biosensors stored at 4°C showed that the enzyme was alive after more than three months, and that the biosensor values remain between the permitted limits in the control graphic for all that period. The developed biosensors can be regarded as stable screening tools for CFV in real water samples, meeting the actual need of low cost screening tools for pesticides in water for environmental monitoring and human consumption.

[1] Directive for environmental monitoring of surface water bodies 2008/105/EC
[2] Council directive on Quality waters intended for human consumption 98/93/EC

Keywords: *Pesticide detection, Chlorphenvinphos, surface water analysis, biosensor, acetylcholinesterase*

R-63
MULTI PESTICIDE RESIDUE DETERMINATION IN RED CHILLI POWDER

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Multi pesticide residue determination in Red Chilli powder using QuEchERS sample preparation and gas chromatography tandem mass spectrometry Abstract A fast and sensitive method has been developed and validated for the analysis of 90 pesticides in dried red chilli powder using the QuEchERS sample preparation technique. Sample preparation was optimized in order to eliminate oleoresin pigment and other co-extractives which can cause retention time shifting, affect chromatographic peak shape and cause loss of sensitivity of the target analytes. The graphitized carbon black (GCB) which adsorbs coloured pigments was tried in different ratios with other sorbents including C18 (alkylated silica) and primary secondary amine (PSA) for the effective cleanup of the acetonitrile extracts. The optimized sample preparation method involved extraction of 2 g of sample with 10 mL of water and 10 mL of acidified acetonitrile in the presence of sodium acetate buffer (1.5 g) and magnesium sulphate (6 g). Cleanup of the acetonitrile extract was achieved using 50 mg PSA and 50 mg C18, 7.5 mg GCB and 150 mg of magnesium sulphate. The limit of qu antification (LOQ) for most of the compounds was 0.99 within the calibration range of 0.3–200 ng/mL. The quantification of the residues was performed using matrix matched standards prepared in residue free organic red chilli powder. The recovery at 10, 25 and 50 ng/mL was within 70–120% (n=6) with associated relative standard deviations below 20%, indicating satisfactory intra-laboratory precision. Chromatographic and mass spectrometric parameters were set up using the Agilent Pesticide and Environmental Contaminants Database (G9250AA). Target analyte MS/MS transitions were selected in order to give the best selectivity against the matrix. Cold splitless injection was performed with a retention time locked (RTL) method. Carryover of high volatile matrix compounds in subsequent runs was eliminated using capillary flow technology with mid-column back flushing. The method was applied for the analysis of ten red chilli powder samples and it was found that the method was suitable for the routine analysis of the 90 target compounds evaluated in this study.

Keywords: *Pesticide Residues, GC–MS/MS, Retention time lock*

R-64
LOW RESOLUTION HIGH SPEED TOFMS AS A COST-EFFECTIVE SCREENING TOOL FOR GC–MS PESTICIDE RESIDUES ANALYSIS IN VEGETABLE EXTRACTS

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Modern laboratories involved in food safety analyses, are facing the increasing challenges for multi-residue pesticides determination in complex matrices like food extracts, due to the increasing regulation imposed by the European Commission and other international regulatory organizations, with established Maximum Residue Limits (MRLs) in different food commodities. In case of apolar or middle polar pesticides, the GC–MS approach is widely used. In particular, due to the increasing number of pesticides used in agriculture and the need to reduce the sample preparation step to speed up the overall analysis time, state-of- the- art of multi-residue pesticides analysis is the use of the Triple Quadrupole MS, being more selective for highest number of target compounds and capable to handle extracts with reduced sample clean-up. In spite of the fact that the tandem mass approach achieves unquestionable sensitivity and selectivity and therefore is well accepted and validated as a confirmatory quantitative method, it has some drawbacks in terms of time consuming method development, applicability only for compounds on a target list, overall cost of analysis. Therefore, there is an increasing need to develop screening methods able to detect larger number of organic contaminants and residues, with the possibility to focus more costly analytical efforts on confirmation and quantitation of positive samples only. Time of Flight MS technology has intrinsic features which perfectly fit with the requirements of wide scope screening methods, having the capability to screen several compounds with high sensitivity in a short run, without need of prior knowledge of the contaminants. Scope of the work is to confirm on real samples from different food matrices the applicability of a high speed low resolution GC–TOFMS system for screening purposes, as an affordable analytical tool complimentary to the GC-Triple Quadrupole system, with the aim to match the desire for highest sample throughput, reduced cost per analysis and improved return of investment.

Keywords: *GC–TOF, Pesticides Screening, Food Safety*

R-65
USE OF GAS CHROMATOGRAPHY QUADRUPOLE–TIME OF FLIGHT MASS SPECTROMETRY WITH ATMOSPHERIC PRESSURE CHEMICAL IONIZATION FOR QUANTITATIVE DETERMINATION OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

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Atmospheric pressure chemical ionization (APCI) is a very promising alternative source to common electron ionization (EI). In gas chromatography (GC), EI has been the most widely used in which a relatively strong fragmentation of the molecule happens with this ionization mode. As a result, the most diagnostic ion of the analyte, the (quasi)molecular ion, is often lost and similar spectra may be obtained for analogues of some analytes. Consequently, for detection and identification, reference spectra of target analytes and also retention times are required to distinguish between structural analogues. In this work an APCI source has been used as ionization mode with mass spectrometer based in a high resolution QTOF analyzer coupled to GC in pesticide residue determination with screening and quantification purposes. The extraction procedure was carried out following the modified acetate-buffered version of the QuEChERS method. The non-target acquisition mode of QTOF analyzers with APCI source take the advantage of acquiring data both at low collision energy and at a collision energy ramp (MSE). A qualitative method was previously validated for around 150 pesticides and then applied for the screening of food samples including strawberries, oranges, apples, carrots, lettuces, courgettes, red peppers and tomatoes. The samples, as well as a quality control at 0.05 mg/Kg for each matrix, were extracted and analysed together with a matrix-matched calibration curve ranging from 0.01 to 0.25 mg/L. Firstly, a total of 27 potential candidates to be positive findings were detected, taking into account only the low collision energy function with the presence of one ion with mass error < 5 ppm. After that, 17 out of 27 analytes were satisfactorily identified with the fragmentation information obtained from the high energy function requiring the presence of the fragment ion at the same retention time, with mass error < 5 ppm. In order to check the suitability of the quantitative method, a validation was performed for these positive findings. Therefore, five matrices were selected as representative (orange, apple, tomato, lettuce and carrot) and were spiked at two different levels (0.01 and 0.1 mg/Kg). The extraction procedure was applied and the samples were quantified using a matrix-matched calibration standard using relative responses versus internal standard (triphenylphosphate). Again the presence of one ion at low collision energy function and the fragment ion obtained at high energy function, both with mass errors < 5 ppm, and also the accomplishment of the ion ratios within the allowed tolerances, were required for a reliable identification and confirmation of the studied pesticides. Recoveries obtained were between 70 and 120 % and RSDs were lower than 20 %, with some exceptions. After all, previous data from the fruit and vegetable samples analysed in the first place could be adequately quantified without the need of injecting and analysing them again.

Keywords: *Fruits and vegetables, pesticides, QuEChERS, GC–(APCI) QTOF MS, screening, quantitative validation*

R-66
MULTI-CLASS PESTICIDE DETERMINATION IN ROYAL JELLY BY GAS CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY

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Royal jelly is one of the most important products from beehive because of its nutritional and pharmaceutical properties. This product is usually sold in nutraceutical presentations like dietary supplements. These supplements are gaining importance because they can deliver a concentrated form of a bioactive compound from a food used to enhance health in dosages that exceed those that could be obtained from the normal food. Beehive products, such as royal jelly, could be contaminated by substances, such as pesticides, used in the beehive itself or in the plants where bees collect nectar or pollen. Thus, pesticides like organochlorines, organophosphorus and carbamates are commonly found in bee products. In Europe, Regulation 396/2005 [2] defines pesticides maximum residues limits (MRLs) for every food and feed, including honey and their derivates. This indicates the health problem that could be presented involving the presence of pesticides in royal jelly. Therefore, analytical methods that can offer a reliable determination of pesticides in royal jelly should be considered. This work described a validated method to analyze multi-class pesticide in royal jelly. Sample treatment was performed using solid phase extraction (SPE) with C18 cartridges. Ethyl acetate and n-hexane were used for pesticides elution. Gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC–QqQ–MS/MS) was used to quantify more than 100 pesticides in less than 23 min. The method was validated in royal jelly products (liquid presentations). Performance characteristics, such as trueness (in terms of recovery), precision, linearity, detection limits (LODs) and quantification limits (LOQs) were studied. Recovery values ranging from 70 to 120% and precision (expressed as relative standard deviation (RSD)) less than 20% were obtained for most of the target compounds at three concentration levels (10, 50 and 100 μg kg^{-1}). Linearity was evaluated in the range 5–100 μg kg^{-1}. In order to avoid matrix effects, quantification was performed by using matrix-matched calibration standards. Furthermore, LODs and LOQs were calculated, obtaining values lower than 10 μg kg^{-1}. The proposed analytical methodology would be applied to the analysis of pesticides in commercial royal jelly products in order to ensure food safety in this kind of products.

Keywords: *Royal jelly, pesticides, mass spectrometry, nutraceutical, gas chromatography*

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R-67
GC–QTOF – AN EFFICIENT TOOL FOR TEA SAFETY CONTROL

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Tea is an old worldwide popular commodity. It is valued for its specific aroma and flavor as well as for health-promoting properties. However, under certain conditions tea leaves may contain some contaminants such as pesticides or polycyclic aromatic hydrocarbons (PAHs), which may be present there due to improper drying process of tea leaves. During the recent years, an increasing public concern and scientific investigation have been focused on the presence and control of these contaminants in herbal products of plant teas to assess the potential health hazards more thoroughly. Within this study a new rapid and flexible method for the simultaneous determination of 137 pesticide residues and 16 PAHs in tea samples was developed and validated. A substantial simplification of a sample processing prior to the quantification step was achieved: after addition of water to a homogenized tea sample, transfer of analytes into the acetonitrile layer was supported by the addition of inorganic salts. Bulk co-extracts were subsequently removed by liquid–liquid extraction using isooctane with the assistance of added 20% (*w/w*) aqueous NaCl solution. For identification/quantification of target analytes gas chromatography coupled to A) quadrupole time-of-flight mass spectrometry (GC–QTOF) and B) triple quadrupole (GC–QQQ) method were used. The recoveries for most of the pesticide residues in the range of 54–101% and for PAHs in the range of 64–105% with repeatabilities (relative standard deviations, RSDs) ≤20% were achieved. Under optimised GC–QTOF conditions, most of the pesticide residues gave lowest calibration level ≤0.01 mg kg^{-1}, permitting the control of pesticide residues at the maximum residue levels (MRLs) laid down in Regulation (EC) No 396/2005. For PAHs the lowest calibration levels were in the range of 0.1–0.3 $\mu g.kg^{-1}$.

Keywords: *GC–QTOF, tea, pesticides, PAHs*

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R-68
FAST SPME GC COUPLED WITH HIGH-SPEED TRIPLE QUADRUPOLE MASS SPECTROMETRY FOR THE SIMULTANEOUS ANALYSIS OF UNTARGETED AND TARGETED TEA VOLATILES

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The present investigation is focused on the evaluation of a novel high-speed triple quadrupole mass spectrometer (QqQ MS), under moderately-fast GC conditions. The QqQ MS system is capable of operation under high speed GC conditions, in both full-scan (maximum scan speed: 20,000 amu/sec) and multiple reaction monitoring (MRM) modalities. Furthermore, the QqQ MS system can generate full scan and MRM data simultaneously, also in a very fast manner. Analytes extraction was performed by using headspace solid-phase microextraction (SPME), for different blends of tea. A fast GC–MS/MS method was developed for the: I) qualitative analysis of unknown tea constituents, and II) MRM quali/quantitative analysis of targeted ones, namely thirty phyosanitary analytes. The QqQ MS system generated a satisfactory number of data points/peak for both qualitative and quantification purposes. The level of sensitivity, reached through the MRM application, widely satisfied the requi rements of current regulations.

Keywords: *Fast GC, food analysis, pesticides, SPME, Triple quadrupole MS*

Acknowledgement: *The Project was funded by the "Italian Ministry for the University and Research (MIUR)" within the National Operative Project "Hi-Life Health Products from the industry of foods". Project ID: PON01_01499.*

R-69
VALIDATION OF A LC–MS/MS METHOD FOR BENZALKONIUM CHLORIDE AND DIDECYLDIMETHYLAMMONIUM CHLORIDE IN FRUITS AND VEGETABLES. EVALUATION OF MATRIX–MATCHED CALIBRATION AND STANDARD ADDITION METHOD

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Quaternary ammonium compounds (QAC) are cationic surfactants that contain one hydrocarbon alkyl chain linked to a positively charged nitrogen atom [1]. The most common QAC is benzalkonium chloride (BAC). Didecyldimethylammonium chloride (DDAC) is used as a plant protection product. With Decision 2004/129/EC most of the QAC have not been included in Annex I of Directive 91/414 of the EU which is now repealed by Reg (EC) 1107/2009. Therefore, only DDAC is approved within Reg (EC) 1107/2009. QAC can be enriched in cell membranes of living organisms impairing cell membrane functions. They are commonly used in domestic products, as disinfectants and biocides [1]. Subsequently, their residues can be found in food after the disinfection of processing areas or the application of plant strengtheners containing DDAC [2]. A procedure for their analysis in water and particulate samples [3] was adapted to fruits and vegetables. QAC were analyzed by LC-ESI+–MS/MS. Two transitions were selected for each compound. Two QuEChERs extraction procedures (AOAC 2007.01 and CEN 15662) were tested, being the first one selected. No clean-up step was included because QAC were retained by the tested sorbents (PSA, C18 and GCB). Validation was carried out with three representative matrices (lettuce, orange and avocado) spiked at three levels (0.01 mg/kg, their default MRL, 0.1 mg/kg and 0.5 mg/kg) in three different series. Matrix effect was evaluated with the post-extraction spiked method [4] and produced 20–30% of signal suppression in all matrices. Therefore, quantification with solvent calibration gave incorrect recoveries. Matrix matched calibration (MMC) and the standard addition method (SAM) were studied for compensating matrix effect. Provided that the method gave good recoveries, SAM was also prepared by adding the analytes to an aliquot of sample extract prior to injection [5]. The procedure was validated by pooling the results for the three matrices in order to represent the wide variability of fruits and vegetables. Only values quantified by SAM in extract were not significantly different due to better compensation of matrix effect. This was the selected approach for quantifying QAC residues. It provided an LOQ of 0.01 $mgkg^{-1}$ calculated from the absolute accuracy profiles. This validated method was implemented at SCAV to monitor 108 fruit and vegetable samples belonging to a Swiss campaign in May 2013 (all of them compliant).

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Keywords: *Benzalkonium chloride, didecyldimethylammonium chloride, vegetables, validation, standard addition method*

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R-70
RESIDUE ANALYSIS ON THE CROSSROAD: THE NEED OF NON-TARGET SCREENING IN FOOD AND ANIMAL FEED BY UHPLC-HR-Q-TOF MS AS ALTERNATIVE FOR THE CONVENTIONAL TARGET SCREENING APPROACH

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Presently the prevailing method for determining pesticides, Vet drugs and other legislative relevant contaminants in food and animal feedstock is a targeted approach using LC/MS/MS or GC/MS/MS. The definitions of methodologies and its target compounds are mainly based on the juridical requirements as well as customer requests on known residuals of which relevance is mostly based on historical experiences. New active substances, contaminants and substances, not in the mainstream of public awareness, are often overlooked hitherto the inherent methodology of targeted analytical technologies. In an increasingly globalised World with foodstuff and packaging materials from a multitude of suppliers and origins, a targeted screening will inherently risk of omitting potential unwanted and toxic contaminants. The goal of our development project was to develop a sensitive non-targeted screening methodology for determination of pesticides, Vet Drugs and other contaminants in Food and Animal feedstock using high resolution UHPLC and QToF MS technology. The Screening Detection Limits (SDL) for these substances should be below the MRL and/or no more than 5 % false negatives should be accepted. After intensive investigations on routine screening equipment in the high resolution mass spectrometry range, the selection was made on a UHPLC QToF (Acquity UPLC and Xevo G2-S QToF, Waters Corporation). This UPLC-MS system uses the so-called MSE technology with a large library which can rapidly identify and quantify targeted compounds using a sophisticated software approach. Furthermore and in the same sample run, unknowns can also be isolated and possibly identified using compound-specific characteristics, such as accurate mass, the identification of adducts, fragmentation patterns, retention time and isotope ratios. The validation of this non-targeted screening method was carried out using the current SANCO document 12495/2011 and also the Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines (Community References Laboratories Residues) and was successful for more than 600 selected pesticides, 200 Vet Drugs and 250 other contaminants of relevance. The SDLs were in the range from 1 to 10 µg/kg. This novel approach in analytical work will in the near future replace the tandem MS systems, which will be continued in the usage for confirmatory analytical work.

Keywords: *Non Target Screening, Unknown Screening, HR QToF-MS, Pesticides, Veterinary Drugs*

RESIDUES

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VETERINARY DRUGS ET AL.

(S-1 – S-54)

S-1
DETERMINATION OF POLAR VETERINARY DRUGS AND PHARMACEUTICALS IN FOOD SAMPLES OF ANIMAL ORIGIN BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY (HILIC–MS/MS)

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Veterinary drugs are widely used in dairy livestock for therapy and prophylaxis of diseases, or as growth promoters. Their extended and excessive use may result in drug residues being present in food, causing potential development of antibiotic-resistant bacteria. Moreover, general pharmaceuticals for human medication are continually introduced into the environment as a result of industrial and domestic use and they are considered as widespread emerging contaminants with potential to enter the food chain. All these reasons make the control of veterinary drug and pharmaceutical residues an important measure in ensuring consumer protection. Hydrophilic interaction liquid chromatography (HILIC) proved to be very effective for the retention and separation of a wide range of polar and hydrophilic compounds compared to reversed phase and ion-pair separation, providing high sensitivity with MS analyzers. In this work a method was developed for the simultaneous determination of polar veterinary drugs and pharmaceuticals, belonging in different classes (aminoglycosides, sulfonamides, quinoxalines, antidiabetic drugs and others) using HILIC–MS/MS. A thorough ionization study of the compounds was performed under HILIC conditions in order to achieve maximum sensitivity and significant differences in the abundance of the precursor ions of the analytes were revealed depending on the constitution of the mobile phase tested. Moreover, a comparison of different stationary phases (aminopropyl- and zwitterionic phases) was performed in order to study the retention of the analytes and the chromatographic separation. The developed method was fully validated in milk, egg and animal tissue samples, achieving good performance criteria and applied in the analysis of food samples of animal origin.

Keywords: *Veterinary drugs, pharmaceuticals, HILIC, LC–MS/MS*

Acknowledgement: *This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.*

S-2
NATURAL WAYS OF ANTIBIOTICS' INGRESS INTO THE PRODUCTS OF ANIMAL ORIGIN

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During the elaboration of mass spectrometric method for the determination of banned antibiotic chloramphenicol (CAP) in dairy products we have detected its residues (0.058 μg kg^{-1}) in cow's milk used as blank control (LOQ of method 0.050 μg kg^{-1}). According to the reliable and verified information, these animals have not been treated with any veterinary drugs over a long period of time and their dietary intake consisted only of roughage and hay. The samples of hay, fed to the animals, have been examined by means of liquid chromatography tandem mass spectrometry method using precursor CAP ion *m/z*=321 and two product ions, viz. *m/z*=257 and *m/z*=152. The analysis has confirmed that tested hay samples contained 0.05–0.82 μg kg^{-1} of CAP. As a result of hay botanical composition study, the presence of individual plants of wormwood Artemisia vulgaris containing 0.21–1.95 μg kg^{-1} of CAP has been established. Further examination of Artemisia plants gathered in the haying region (Artemisia vulgaris) and medicinal plants (A.vulgaris, A. Absentis) purchased from local pharmacy has confirmed CAP content in Artemisia plants at the concentration level of 0.08-1.95 μg kg^{-1}. The study of CAP localization in different parts of Artemisia plants and root soils gathered from the pasture has showed that maximal CAP concentration appeared to be in caulis and leaves, whereas minimal CAP content has been detected in soil from plant's rootosphere, viz. 8.12 and 0.09 μg kg^{-1} respectively, which is partly confirmed by the available data. Preliminary analyses of the obtained data have enabled the suggestion about the ability of overground parts of Artemisia plants to accumulate chloramphenicol, produced by soil bacteria Streptomices. In order to establish possible effect of the discovered phenomenon on safety parameters of dairy products we have carried out the retrospective analysis of the results of milk powder and other milk products control, performed in the institute over 2002–2012. According to the current legislation, the regulatory limit of chloramphenicol residues in food of animal origin is established by minimum required performance limit (MRPL) 0.3 μg/kg. Previous analysis of the data has been carried out taking into account only the samples with CAP content higher than 0.3 μg/kg, in consideration of the method error. Thus it was not possible to estimate completely the level of milk products contamination with CAP residues. When carrying out reanalyses we have also taken into consideration the data, which exceeded mean values of the matrix blank (+2σ), obtained on the method validation. As a result of the statistical analysis carried out, it has been established that among 2452 powder milk samples containing >0.3 μg/kg CAP there were 1205 samples with ascertained CAP concentration at the level of 0.05–0.15 μg kg^{-1}.

Keywords: *Chloramphenicol, residues, Artemisia, feed, milk*

S-3
DETECTION OF ILLEGAL USE OF ANTIBIOTICS IN POULTRY BY FLUORESCENCE MICROSCOPY

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Tetracyclins are antibiotics registered for use in, amongst others, livestock. They can only be used therapeutically when prescribed by a veterinarian, and a withdrawal period has to be applied to prevent presence of antibiotics in meat for consumption. However, tetracycline residues are regularly found in retail broiler meat. This can have several reasons; a too short withdrawal time, illegal use in sub-therapeutic dosages as growth promoter, or recirculation in the diet via the bedding. The possibility to use fluorescence microscopy of the antibiotic oxytetracyclin (OTC) in cross-sections of tibia bone is studied to distinguish between untreated broiler chicken and several treatment strategies. The cross-sections of the tibia of untreated broilers show no fluorescence, while the tibia of treated broilers show clear fluorescent patterns. A therapeutic treatment with a short withdrawal time results in fluorescent rings around the Haversian channels. The longer the withdrawal time after treatment, the further the tetracylins will diffuse and dilute in the bone. The tibia of sub-therapeutically treated broilers show a diffuse, evenly distributed fluorescence pattern. The patterns give a good indication of the use of this type of antibiotics. Therapeutic use of tetracyclins is only allowed when the legally required withdrawal time is maintained to ensure that the residue levels in the meat are below the Maximum Residue Level (MRL). Control of the medicine administration of the farm and additional LC–MS/MS analysis can be used to detect allowed or illegal treatment. Analysis of the broiler meat showed that after sub-therapeutic treatment the MRL was exceeded, while the OTC levels in meat of therapeutically treated broilers (complying with the required withdrawal time) remained below MRL. To test the method with actual products, the tibia bones from drumsticks bought at an organic butcher and a supermarket were investigated. The supermarket products show a diffuse fluorescence over the whole bone surface that can be caused by sub-therapeutic treatment. The profiles of the bone from the organic butcher show more resemblance either with the blank or with the profiles of the therapeutically treated broilers with a short withdrawal time.

Keywords: *Antibiotics, tetracyclines, fluorescence, microscopy*

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S-4
FAST EXTRACTION AND SENSITIVE DETECTION OF LOW LEVELS OF CHLORAMPHENICOL IN SHRIMP USING Z-SEP+ SORBENT IN QUECHERS SAMPLE PREPARATION APPROACH AND ANALYSIS BY LC–MS/MS

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Chloramphenicol is a broad-spectrum antibiotic that has been used to treat several disease conditions in domestic animals and farmed fish. However, due to its potentially harmful side effects in humans, such as aplastic anemia and hypersensitivity reactions, it has been banned from use in animals for food production. In many countries, the tolerance for chloramphenicol in food products is zero. Fishery and aquaculture products are usually subject to tests to ensure the absence of chloramphenicol residues. The low detection limit of 0.1–0.3 µg/kg is set for chloramphenicol in shrimp. Analytical confirmatory methods for chloramphenicol usually include time-consuming cleanup steps prior to LC–MS/MS analysis. Recently, new SPE sorbents, Z-Sep and Z-Sep+ became available. These were applied previously to preparation of fish tissue samples for analysis of pesticides, flame retardants and PAHs in a fast sample extraction and cleanup approach called QuEChERS. Using a QuEChERS like approach, both Z-Sep and Z-Sep+ sorbents were tested for the cleanup of extracted shrimp samples prior to analysis of chloramphenicol by LC–MS/MS. The presented work will detail the fast sample preparation method using Z-Sep+ sorbent that resulted in the best overall recoveries and method performance. The sample cleanliness was compared to that from the “dilute-and-shoot” approach and found to be superior when using Z-Sep+ cleanup. The cleaner samples resulted in better method ruggedness, less instrument maintenance and better analytical performance. In addition, low quantitation limit of 0.1 ng/kg was easily reached by the proposed method.

Keywords: *Chloramphenicol, QuEChERS, extraction, cleanup, shrimp*

S-5
DEVELOPMENT OF A SIMPLE GENERIC BEEF TISSUE SAMPLE PREPARATION METHOD FOR APPLICATION TO MULTIPLE IMMUNOASSAYS FOR THE DETECTION OF ZILPATEROL AND RACTOPAMINE

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Introduction. 2013 reports estimate global beef production at 57.5 million tons. Ractopamine and zilpaterol are beta-adrenergic agonists that can be used as growth promoters and have been shown to increase muscle growth and decrease fat deposition in animals. Of particular concern is the use of beta agonists prior to slaughter as this poses a risk to the consumer. Some countries have imposed import restrictions on certain meat products and banned ractopamine in beef, deeming it unfit for human consumption whereas other countries have deemed meat from animals fed with ractopamine as safe for human consumption. Due to global differences related to the use of these veterinary drugs, it is vitally important to screen for their presence to adhere to global regulatory requirements and therefore prevent possible import/export issues.
This study reports on the development of a reliable, generic, fast and simple beef tissue sample preparation method for the detection of zilpaterol and ractopamine on two immunoassay based testing platforms: enzyme-linked immunosorbent assay (ELISA) and biochip array.
Method. The sample extraction method involves the use of homogenised beef tissue (5 g) the addition of acetonitrile:methanol, centrifugation, evaporation, and reconstitution (>40 samples can be prepared in just over 2 hours). The samples are now ready for application to the microtitre plates or the biochip platform. For the determination of ractopamine and zilpaterol competitive immunoassays were employed. In the application to ELISA following incubation and washing steps, enzyme substrate was added. Measurement of the optical density was carried out at 450 nm. In the application to the biochip platform, detection took place using digital imaging technology on the semi-automated Evidence Investigator analyser.
Results. Application of this generic sample preparation method to the ELISA platform found that the limit of detection (LOD) of the ractopamine assay was 0.1 ng/g (assay range: 0–1 ng/ml), with sample recovery ranging from 73 to 92%. The zilpaterol assay presented a LOD value of 0.08 ng/g (assay range: 0–4ng/ml) and the recovery range: 75–108%. For both ELISA assays the intra-assay precision was <10%. In the application to the biochip platform, the ractopamine assay presented a LOD of 0.1ng/g (assay range: 0–5ng/ml), recovery range: 74 to 110%. The zilpaterol assay exhibited a LOD of 0.08ng/g (assay range: 0–5 ng/ml) and recovery range: 94–125%. For both biochip assays the intra-assay precision was <10%.
Conclusions. The results show applicability of the generic sample preparation method of beef tissue to the detection of zilpaterol and ractopamine on two immunoassay based testing platforms: ELISA and biochip array. Both immunoassay based platforms exhibited high sensitivity and excellent recovery. Moreover, this sample preparation method is reliable, simple and rapid. This will aid in the more rapid screening of both substances in beef tissue samples.

Keywords: *Beef sample preparation, beta agonists, immunoassays, ractopamine, zilpaterol*

S-6
ANTIBIOTIC RESIDUE CONTROL IN FRANCE: CONTRIBUTION OF MASS ANALYTICAL METHODS TO IDENTIFICATION OF RESIDUES

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Microbial growth inhibition tests are widely used as the screening approach for the detection of antibiotic residues in meat. In France, part of the antimicrobial residue control program for cattle, porcine, ovine, caprine and equine species is currently implemented as follows: muscle samples are analyzed by inhibition tests in field laboratories and the positive samples sent to the National Reference Laboratory for confirmation by LC–MS/MS procedures. A LC–MS/MS method for the screening of antibiotic residues in muscle was developed and validated according the 2002/657/EC Decision by the National Reference Laboratory (NRL) for Veterinary Drug Residues. It allows getting information about the presence and the identity of antibiotic substances (61 analytes among the main antibiotic families used in veterinary medicine) in muscle. The positive samples are then quantified by specific LC–MS/MS. This strategy is now implemented for two years at the level of 10 fi eld laboratories using different LC–MS/MS systems (triple quadrupole, ionic trap). Before this transfer, a training session was organized by the NRL allowing the operators to take the method in hand and a collaborative study was planned. Since 2011, this strategy was in application for the French antibiotic control plan. An outcome of the first results and a comparative study with biological screening results will be presented.

Keywords: *Antibiotics, residues, screening, LC–MS/MS*

S-7
THE POTENTIAL OF ATMOSPHERIC SOLIDS ANALYSIS PROBE (ASAP) IONISATION MASS SPECTROMETRY FOR RAPID IDENTIFICATION OF ANABOLIC STEROID ESTERS

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The use of anabolic substances for livestock fattening has been prohibited in the European Union for more than 20 years (Directive 96/22/EC). Nevertheless, in a crisis sector, their use is still suspected regarding potential benefits in terms of income. Among the growth promoters administered, anabolic steroid esters are the most commonly used due to their higher bioavailability and their longer lasting effect. Efficient control of their administration is based on their detection by LC–MS/MS in biological matrices such as plasma or hair. Such protocols however are time consuming in terms of sample preparation and detection. In this context, the present study aims to provide a new rapid, efficient and multiresidue confirmatory strategy thanks to the Atmospheric Solids Analysis Probe (ASAP) technology. The ASAP source is a method used for rapidly analysing liquid or solid materials based on atmospheric pressure ionisation. In this technique, sample is directly introduced into the mass spectrometer; compounds are vaporized thanks to the heated nitrogen nebulising gas and ionised by a corona discharge. Firstly, twenty three steroid esters of testosterone, estradiol, boldenone and nandrolone were analyzed with this technique to investigate the mechanism of ionisation both in positive and negative mode. ASAP analysis generally results in the formation of the pseudo molecular ion. Collision induced dissociation (CID) of the pseudo molecular ion often led to specific ions both of the steroid in its native form and of the esters. These results allowed developing a precursor ion scan method to identify both known but also any unknown steroid esters. Finally, we developed and validated an isotopic dilution ASAP method in SRM mode for the quantification of these compounds. Reproducibility, linearity, sensitivity and matrix effect were evaluated in hair and serum as well as in oily preparations.

Keywords: *Chemical Food Safety, Steroid esters, ASAP, Ambient Mass Spectrometry*

S-8
FLUOROQUINOLONE RESIDUES DETECTION BY LC–MS/MS/MS IN TREATED TURKEYS

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A confirmatory method for five fluoroquinolones (FQs), enrofloxacin, ciprofloxacin, difloxacin, sarafloxacin and flumequine was developed using liquid chromatography coupled to mass spectrometry (LC–MS/MS/MS) to quantify FQ concentrations in blood, liver, kidney, muscle, skin+fat, lung and cecal content obtained from farm treated turkeys, for PK/PD studies. Biological matrices behave differently when extraction procedures are applied and it is critical to find an efficient and robust sample pretreatment for matrix clean-up and analyte extraction. Instead of the clean-up step by SPE that requires large amount of solvent and is time consuming [1], for the extraction of FQ residues, the QuEChERS (Quick Easy Cheap Effective Rugged Safe) methodology was extended to all matrices of animal origin, with the exception of blood where only acetonitrile addition was applied. The LC separations were performed on a C18 Kinetex column (100 × 2.1 mm, 2.6 µm, Phenomenex, CA, USA) with gradient elution using ammonium acetate solution (10 mM, pH 2.5) and methanol containing 0.1% formic acid [2]. Mass spectrometric identification was done using a LTQ XL ion trap (Thermo Fisher Scientific, CA, USA), equipped with a heated electrospray ionization probe, operating in positive ion mode. The identities of each FQ were confirmed by monitoring via MS^3 the ratios of two prominent product ions, previously selected. The method was validated meeting the European Legislation (decision 2002/657/EC) determining selectivity, linearity response, trueness, precision (repeatability and between-day reproducibility) decision limits, detection capability and recovery. All data, obtained using turkey blank matrices spiked with standard solutions containing all FQs, were within the required limits established for confirmatory methods. Limits of quantification for all FQs ranged from 12.5 to 118.8 µg/kg, by far lower than the Maximum Residue Limit (MRL) established by European Union, for all FQs, in turkey tissues (100–1900 µg/kg). The QuEChERS technique adopted for biological matrices did not require further clean-up step and the extraction rates ranged from 80% to 105% for all FQs. The method was simple, fast, robust and suitable for the identification and quantification of FQ residues in tissues and cecal content as confirmed by data obtained from incurred samples of turkeys treated for therapeutic purposes.

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Keywords: *Fluoroquinolones, LC–MS/MS/MS, residues, QuEChERS, biological matrices*

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S-9
SIMULTANEOUS DETERMINATION OF GLUCOCORTICOIDS IN LIVESTOCK PRODUCTS WITH UPLC–ESI–MS/MS

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A new method for the simultaneous determination of five glucocorticoids (betamethasone, dexamethasone, prednisolone, methylprednisolone, and flumethasone) in livestock products was established. Samples were effectively extracted using OASIS HLB solid phase extraction with acetonitrile. Chromatographic separation was achieved using C18 column and negative electrospray ionization mass spectrometry was performed in the Multiple Reaction Monitoring mode for the effective quantitation and qualification of glucocorticoids. Acetonitrile and water (0.1% formic acid) were used as mobile phase and additive for effective electrospray ionization, and gave good chromatographic separation and mass spectrometric sensitivity. The limit of detection (LODs) and the limit of quantitation (LOQs) in spiked blank samples depending on types of matrix and pharmaceuticals were ranged from 0.2 to 1.0 μg/kg and 0.8 to 3.4 μg/kg, respectively. And the recoveries were between 85.5 to 119.6%. The established method showed good recoveries, accuracies, precisions and fast sample preparation and it will be applied to assay of glucocorticoids residues in livestock products.

Keywords: *Glucocorticoids, livestock products, LC–MS/MS, residual analysis*

S-10
QUADRUPOLE-ORBITRAP STEROID ANALYSIS IN BOVINE URINE

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In Europe hormones are illegally used as growth promoters in cattle. Detection of abuse of growth promoters usually takes place through directed chemical analysis of urine samples on a limited list of substances using triple-quad Mass Spectrometers. Until now, attempts to detect growth promoters using TOF/Orbitrap technologies in urine proved to be problematic due to the low concentrations of these compounds present in samples of urine. Also, these Mass-Spectrometers are limited to screening analysis and for confirmation analysis traditional triple-quad confirmatory analysis are necessary. Due to increased sensitivity and selectivity of the latest generation Quadrupole-Orbitrap Mass Spectrometers, growth promoters in samples of urine can be detected and confirmed at relevant concentration levels. Urine samples from bovine animals were spiked with a number of steroids on different concentration levels. These samples were measured using the different scan modes of the Q-Exactive. In this study the sensitivity, specificity and confirmation capabilities of the Q-Exactive in a representative set of samples of urine are presented.

Keywords: *LC-MS, High Resolution Accurate Mass, veterinary drugs*

S-11
PLACKETT-BURMAN DESIGN FOR THE EXTRACTION OF THYREOSTATS WITH LC-MS^2 DETECTION IN BRASSICACEAE FEEDS

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Thyreostats, such as thiouracil (TU), have been banned since many years in the European Union for their role in the illegal fattening of livestock and the teratogenic and carcinogenic effects of residues thereof in animal products upon human consumption. In recent years, several studies have shown that thyreostats even though considered as synthetic, may also have a natural origin. The study by Pinel et al. (2006) gave the first insight by proving the administration of Brassicaceae crops caused TU excretion in bovine urine. At that time, TU could not be recovered from the administered feed. These findings led to believe the presence of a precursor in the crops could be responsible for the endogenous TU formation. Shortly after, Vanden Bussche et al. (2011) demonstrated that the use of the plant enzyme myrosinase enabled TU detection in Brassicaceae food and feed. Hereupon several animal feeds, which were suspected of containing TU, were analysed on their TU content by myrosinase hydrolysis. The recovered TU concentrations fluctuated around 10 ppb. Since the daily feed intake by livestock lies in the order of kilograms, the effect of this recurring intake could be the probable source of the low TU levels in livestock urine. Nevertheless, the analysis of animal feed proves to be a difficult task and at the moment no robust and broadly applicable food and feed extraction procedure is available yet. Therefore, the aim of this study was to develop a reproducible, routinely applicable sample preparation procedure for TU in a variety of Brassicaceae feeds followed by mass spectrometric detection. For this, the approach of a statistical Plackett-Burman (PB) design was preferred over a laborious one-at-the-time optimization. The PB model gives the possibility of simultaneously analysing various parameters and their mutual interactions by a reduced number of extractions. A literature study resulted in a subset of relevant parameters to be evaluated e.g. variables related to the feed (e.g. amount), the derivatization step (e.g. volume of the agent), the myrosinase hydrolysis (e.g. volume) and the final extraction steps (e.g. solvent volumes for liquid/liquid extraction and solid phase extraction). Each parameter was matched with two alternatives and inserted into the model. After sample preparation and LC separation, the detection of TU was performed by an LTQ™ (Thermo Electron, San Jose, US) ion trap mass spectrometer. The LC-method included a 50:50 gradient of 0.5% acetic acid in water and methanol at a 0.3 mL min^{-1} flow rate, which sufficed to the separation of TU over a C18 Symmetry column. Detection was then performed in the negative ion mode with a heated electrospray ionisation source. For this application, the ion trap has many desired assets, like the reduction of matrix interferences and the potential of structural elucidation of the TU precursor by MSn in the future, which match the challenges of these complex matrices.

Keywords: *Thyreostats, Brassicaceae feeds, Plackett-Burman extraction optimization, LC–MS^2*

S-12
DEVELOPMENT AND VALIDATION OF A METHOD FOR DETERMINATION OF FIVE BETA-AGONISTS IN BOVINE URINE WITH MOLECULAR IMPRINTED POLYMER (MIP) SPE AND DETECTION AND QUANTIFICATION WITH HPLC–MS/MS

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Beta-agonist drugs are commonly used in veterinary and human medicine for therapeutic purposes as bronchodilators. In animal production, they may also be employed as growth-promoters, since these molecules are able to increase muscular tissue deposition. Their use as anabolic agents is considered illegal in many countries, and therefore there is a need for analytical methods to determine the presence of beta-agonists residues in animal derived products. In order to meet this requirement, a method for simultaneous determination of the beta-agonists cimaterol, clenbuterol, ractopamine, salbutamol and zilpaterol in bovine urine was developed and fully validated. Sample extraction was carried out using class-selective MIP cartridges and the analytes were quantified by HPLC–ESI–MS/MS operating in positive MRM. Level of interest was established as 1.0 ng/mL for all analytes. The validation comprehended essays for the evaluation of selectivity, linearity, repeatability, reproducibility and robustness. Decision limits and detection capabilities were determined. Values for CCalpha ranged from 0.14 to 0.32 ng/mL and from 0.23 to 0.49 ng/mL for CCbeta.

Keywords: *Beta-agonist, validation, veterinary drug residues*

Acknowledgement: *MAPA, CNPq*

S-13
MINIATURIZED, ULTRA-FAST AND HIGH SENSITIVE TECHNIQUES COMBINED WITH NEW SAMPLE TREATMENTS FOR THE MONITORING OF CEPHALOSPORINS, QUINOLONES AND NITROIMIDAZOLES IN FOODSTUFFS OF ANIMAL ORIGIN

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The extensive use of antibiotics in veterinary medicine represent a potential hazard for human health, since they can produce residues in foods derived from animals. European Union (EU) has established maximum residue limits (MRLs) for different antibiotics in foodstuff of animal origin through Commission Regulation N. 37/2010, including cephalosporins (CPs) and quinolones (QNs), which are among the most common antimicrobials used in both, human and veterinary medicine. Genotoxic, mutagenic and carcinogenic effects have been attributed to other antimicrobial compounds, 5-nitroimidazoles (5-NDZs), so their utilization has been forbidden in animals intended for human consumption in Europe, USA and China. In spite of the above-mentioned Commission Regulation, which prohibits the presence of these residues in foods derived from animals in EU, there are currently alerts about the illegal use of these substances in veterinary practice (Rapid Alert System of Food and Feed (RASFF) portal). For all these reasons, new analytical methods based on the use of miniaturized (micellar electrokinetic capillary electrophoresis [MEKC] or capillary HPLC) and ultra-high resolution (UHPLC) techniques, combined with strategies to increase sensitivity, such as on-line preconcentration (cation-selective exhaustive injection and sweeping in MEKC) or laser induced fluorescence (LIF) detection are proposed in this communication, for the control of QNs, CPs and 5-NDZs. Also, alternative sample treatments for the extraction of these compounds in different matrices (beef and pork muscle, eggs and different fishes, such as sea bass, trout, sturgeon and panga) have been developed. QuEChERS methodology, salting-out assisted liquid-liquid extraction (SALLE) or solid-phase extraction (SPE) are discussed. In all cases, the proposed methods were characterized for the different matrices, obtaining limits of detection (LODs) lower than MRLs established by EU and with satisfactory recoveries and precision. The proposed methods are simple, with minimum steps and effective for cleaning-up of these complex samples, being suitable alternatives for routine analysis.

Keywords: *Cephalosporins, nitroimidazoles, quinolones, capillary electrophoresis, UHPLC*

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S-14
SIMULTANEOUS DETECTION OF THIAMPHENICOL, FLORFENICOL AND FLORFENICOL AMINE BY IMMUNOBIOSENSOR

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Florfenicol (FF) and thiamphenicol (TAP) are synthetic broad-spectrum antimicrobials belonging to the class of drugs known as amphenicols. They have been extensively used in veterinary medicine because of their effectiveness against a wide range of enteric bacteria including Salmonella typhi and Escherichia coli as well as anaerobic and gram positive aerobic bacteria. They have, however, been shown to display haematological toxicity and so the European Commission (EC) has established maximum residue limits (MRLs). A MRL of 50 μg kg^{-1} has been established for TAP in the tissues of all food producing species while the FF MRL relates to the sum of FF and its major metabolite florfenicol amine (FFA) and has been established as 300 μg kg^{-1} in bovine and ovine kidney, 500 μg kg^{-1} in porcine kidney and 100 μg kg^{-1} in avian muscle. To monitor for non-compliant concentrations of such compounds it is essential that robust and reliable analytical methods are available to allow detection of their residues in samples taken from food producing animals. The implementation of multi analyte screening assays is particularly favourable for regulatory testing laboratories with respect to the numbers of samples that can analysed for multiple targets and the associated savings regarding time and cost. In the present study a multi-residue immunobiosensor (GE Healthcare) based screening assay was developed. A stable biosensor chip surface was prepared and utilized in combination with a simple extraction procedure to allow the detection of FF, FFA and TAP at half their respective MRLs in a variety of species and matrices. An experimental trial was undertaken to evaluate the effectiveness of the developed method when applied to incurred tissue. Groups of broiler chickens were treated with therapeutic doses of either FF (20 mg kg^{-1} bw per day) or TAP (50 mg kg^{-1} bw per day) and the depletion of each drug in tissue was assessed over the course of the withdrawal period. Data from the assay validation including the range of species and matrices analysed and from the experimental trial will be presented.

Keywords: *Thiamphenicol, Florfenicol, Florfenicol amine, Immunoassay, Biosensor*

S-15
THE MANIPULATION OF HAPTEN AND CHOICE OF IMMUNOASSAY FORMAT TO ALLOW THE SIMULTANEOUS DETECTION OF THIAMPHENICOL, FLORFENICOL AND FLORFENICOL AMINE BY A SINGLE ANTIBODY

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Chloramphenicol (CAP) is a broad spectrum antibiotic that has been banned from use in food producing animals due to serious health risks to the consumer. Thiamphenicol (TAP) and florfenicol (FF) are structural analogues of CAP with similar antibacterial activity but without the associated severe adverse effects. However, the EU commission has established maximum residue limits for TAP and FF in a range of target tissues, with the marker residue for FF being the sum of FF and its metabolites measured as florfenicol amine (FFA). Immunoassays employing specific antibodies, that can bind target compounds or families of compounds, have been used as analytical techniques for the detection of veterinary drug residues for the past three decades. While an antibody to CAP may be capable of binding TAP and FF it has been shown that it will not be able to bind FFA sufficiently to allow detection at the required level. Therefore antibodies are needed that can collectivel y bind FF, FFA and TAP. The current study employed a number of chemical conjugation techniques using TAP, FF and FFA to prepare a range of immunogens for antibody production as well as the corresponding enzyme labels for use in direct ELISA format. Results will be presented to show which conjugation techniques produce the most sensitive antibodies to allow detection of all three targets and what effects on sensitivity and specificity can be observed when changing from a homologus to heterologous assay.

Keywords: *Thiamphenicol, Florfenicol, Florfenicol amine, Antibody, Immunoassay*

S-16
FLOW CYTOMETRIC SCREENING ASSAY FOR SIMULTANEOUS DETECTION OF ANTIBIOTICS AND MELAMINE RESIDUES IN MILK

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Antibiotics are widely used for the treatment and prevention of bacterial diseases in dairy cattle. A specific indication for administrating such antibiotics is infectious mastitis. The risks due to the presence of antibiotic residues in milk were quickly identified as well by the health authorities as by industries. The European Commission therefore established Maximum Residue Limits (MRL) for these antibiotic families in milk. Melamine, mainly used in the plastic industry, has been shown to be illegally added to food products in order to increase the apparent protein content. Melamine presence has led to important human health damages. Therefore the Codex Alimentarius Commission and the European Commission have also fixed a maximum level of Melamine in milk. With the aim of ensure consumer safety and regulatory compliance, there is an increasing importance to develop sensitive, rapid, multiple, high-throughput and cost-effective methods for simultaneous determination of residual veterinary drugs and adulterant in milk. In this study, we report the development of a new multiplex flow cytometric affinity assay for the simultaneous detection of the main antibiotics involving at least Tetracyclines, β-lactames, sulfonamides, macrolides, aminoglycosides, lincosamides, polymyxin and (fluoro)quinolones as well as melamine residues in milk. This format of tests is rapid, easy and detects the main antibiotic compounds at concentrations relevant to industries and authorities.

Keywords: *Antibiotics, Melamine, Milk, Multiplex, Flow cytometric assay*

Acknowledgment: *EUROSTARS programme, EUREKA and the Walloon Region*

S-17
E-READER: NEW DEVICE FOR DETECTION OF ANTIBIOTIC RESIDUES IN FOOD

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Analytical tools for routine analyses of antibiotic residues should be reliable, simple and cost-effective. E-reader, a new device that requires just 5 minutes handling, after sample extraction, has been recently developed to screen antibiotic residues. This device can be applied to different screening methods. The system combining Explorer 2.0 with e-reader for screening of antibiotic in meat is presented here. Explorer 2.0 is a microbial test based on the inhibition growth of G. stearothermophilus. The test contains bacteria spores and a pH indicator. When the assay is incubated at the optimal temperature, spores germinate and cells grow producing acid, changing the agar pH and thus the colour. Explorer 2.0 is incubated at 65°C by e-reader that also monitors the colour change and stops the assay automatically. Qualitative results (positive or negative) are displayed on the e-reader screen. The new system, Explorer 2.0 + e-reader, has been validated following the ISO 13969 guidelines. The LODs (Limit of Detection) for 12 different molecules have been determined. Results produced by e-reader were compared with a visual reading interpretation. The system can detect the evaluated antibiotics at or below the European MRLs. Explorer 2.0 + e-reader makes the antibiotics testing very simple as the user does not need to control the assay time neither to interpret the results

Keywords: *Antibiotic residues, meat, screening testing*

S-18
DEVELOPMENT OF RAPID ANALYTICAL METHOD OF SILDENAFIL ANALOGUES IN HEALTH FUNCTIONAL FOODS USING LIQUID CHROMATOGRAPHY–QUADRUPOLE TIME OF FLIGHT MASS SPECTROMETRY

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The scale at which erectile dysfunction(ED) medicines are obtained outside of the official health system and possibly exceeds legitimate sales. The problem is becoming more complex, as concealed, structurally modified analogues are increasingly being used. Therefore, in this study simple, rapid, sensitive and specific liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry(LC/Q-TOF) method was developed for qualitation and building a database of sildenafil analogues in health functional foods, a new selective and reversible phosphodiesterase 5 inhibitor. Chromatographic separation was performed using Waters XDB C18 column (150 mm × 2.1 mm, i.d., 3 μm.) with a mobile phase consisting of 0.1% formic acid and 0.1% formic aicd in acetonitrile at a flow rate of 0.3 ml/min over a total run time of 25 min. The mass spectra of more than 50 illegal sildenafil analogues were measured using an Agilent 6530 instrument with positive and negative electrospray-ionization(ESI), selection of the protonated or deprotonated molecules $[M+H]+$ or $[M-H]-$ by the quadrupole, and collision induced dissociation(CID) with nitrogen as collision gas at CID energies of 5, 10, 15, 20, 25, 30, 35 and 40 eV.

Keywords: *Sildenafil analogues, LC–QTOF, health functional foods*

S-19
OCCURRENCE OF MACROCYCLIC LACTONES IN DAIRY PRODUCTS FROM BRAZILIAN MARKET

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The avermectins and milbemycins belong to a family of compounds called macrocyclic lactones (ML) and are highly used as anti-parasitic agents in the treatment of cattle for control of gastrointestinal nematodes, ticks and myiasis. In Brazil, there are five substances (ivermectin, abamectin, doramectin, eprinomectin and moxidectin) registered for bovines. The use of these compounds may bring benefits to the cows but indiscriminate use might result in the presence of residues in milk and dairy products. In this context, the objective of the study was to validate an analytical method for determination of five ML in dairy products and verify the occurrence of these compounds in milk and yogurt available in the Brazilian market. The method involved QuEChERS sample preparation, derivatization and determination by high performance liquid chromatography with fluorescence detection. The method was validated using organic samples of milk and yogurt for the following parameters: linearity, precision, accuracy, repeatability and limits of detection (LOD) and quantification (LOQ). For yogurt, the method showed good linearity within the range of 0–0.025 mg/kg (r^2 > 0.997). Average recovery, performed at three different levels varied from 88% to 109% (RSDs < 14%). The method provides LOD ranging from 0.0012 to 0.0017 mg/kg and the LOQ was established according to the lower spike level used (0.0025 mg/kg). For milk, all ML showed linearity at the concentration range of 0–0.04 mg/L (r^2 > 0.997), with recoveries between 93 and 114% (RSDs< 9%). The values for LOD ranged from 0.0001 to 0.001 mg/L, and similarly for yogurt LOQ was established according to the lower spike level used in the study (0.0002–0.01 mg/L). Repeatability and within-laboratory reproducibility were satisfactory for both matrixes. In order to monitor milks and yogurts marketed in Campinas, SP, Brazil, 13 brands of UHT milk (135 samples), 8 brands of pasteurized milk (103 samples) and 13 brands of yogurt (104 samples) were acquired. A total of 342 samples were analyzed in duplicate for the presence of ivermectin, abamectin, doramectin, eprinomectin and moxidectin. ML was detected (<LOQ) in three samples of pasteurized milk, one of them presented moxidectin and the other two presented traces of ivermectin. No residue of the analyzed compounds was found in UHT milk or yogurt. Results indicate that the consumption of milk and yogurt does not represent a health risk for Campinas population.

Keywords: *Avermectins, QuEChERS, milk, yogurt*

Acknowledgement: *CNPq, FAPESP and CAPES*

S-20
DETERMINATION OF FLORFENICOL AND FLORFENICOL AMINE IN FISH MUSCLE WITH GAS CHROMATOGRAPHY–NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY

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Florfenicol (FF) is a broad spectrum antibiotic which is approved for use in food-producing animals like cattle, porcine and aquaculture to control bacterial diseases. Florfenicol amine (FFA) is the major metabolite of florfenicol and in the Commission regulation (EU) No 37/2010 the maximum residue limit (MRL) is set for the sum of FF and its metabolites measured as FFA. For fish the MRL is 1000 µg/kg and target tissue is muscle and skin in natural proportions. We developed and validated a GC/MS method at the level of MRL to determine FF and FFA in fish samples. Homogenized samples were spiked with internal standards (florfenicol-d_3 and florfenicol amine-d_3) and ethylacetate ammonium hydroxide (10 ml, 98:2) was added. Samples were carefully mixed with a shaker and centrifuged. Extraction was repeated with another 5 ml of extraction solution. Extractions were combined and let to stand overnight in a refrigeration. Next day samples were acidified with acetic acid (2 ml, 5%) and ethylacetate was evaporated off. The remaining water phase was extracted twice with hexane to remove fat. The samples were subjected to solid phase extraction (MCX, 60 mg, Oasis). Cartridges were first conditioned with methanol (3 ml) followed by water (3 ml). Catridges were not allowed to dry between conditioning and sample introduction. Sample solution was eluted through the column and the column was washed with water solution (5 ml). Finally, the analytes were eluted from the column with methanol ammonium hydroxide solution (90:2, 5 ml). The eluate was collected and evaporated to dryness using nitrogen flow. Derivatizing solution was added (100 µl, TMSI) and the samples were heated at 60°C for 1 hour. After cooling to room temperature hexane (250 µl) and water (1 ml) were added. After centrifugation the upper layer was subjected to GC-MS analysis. The average recovery was 107% for FF and FFA and detection capability CCα for FF was 1274 µg/kg and for FFA 1145 µg/kg. The method was applied to incur whitefish meat and skin samples, respectively.

Keywords: *Florfenicol, florfenicol amine, fish*

S-21
GROWTH PROMOTERS IN MILK BASED PRODUCTS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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To provide consumers with the highest possible standard of safety/quality in infant nutrition products, Nestlé Research Centre has developed a multi-residue screening method for 27 Growth Promoters belonging to 5 families in skimmed milk powders and finished infant formula products. A Liquid Chromatography coupled with tandem Mass Spectrometry (LC–MS/MS) was developed. Compounds are detected in both positive and negative ionization modes, with the monitoring of two transitions for each analyte. The 27 compounds are separated using a Phenyl Hexyl stationary phase column within a 25 min run, using acetonitrile and modified water with 5mM ammonium acetate as mobile phases. Sample preparation is carried out by an enzymatic hydrolysis, followed by the QuEChERS method (EN 15662:2008). Rehydrated milk powder is extracted with acetonitrile, followed by partition with $MgSO_4$/NaCl. The extract is then purified by dispersive solid phase extraction with C18 and PSA (Pri mary Secondary Amines) sorbents and evaporated to dryness. The solvent for the reconstitution is water/methanol (70/30; *v/v*), and the final reconstituted extract is filtered before LC–MS/MS injection. Screening performance parameters were validated for 27 target analytes on 67 samples, including individual skimmed milk powder or infant formulae samples with different origins and recipes. The validation method was performed according to the CRLs Guidelines for the validation of screening methods for residues of veterinary medicines; 2010-01-20.

Keywords: *Growth Promoters, milk based products, LC–MS/MS*

S-22
THE ANALYSIS OF COCCIDIOSTATIC AGENTS IN FEED USING UPLC–TANDEM QUADRUPOLE MS

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Coccidiosis is a parasitic disease of the intestinal tract of animals, and spreads from one animal to another by contact with infected feces or ingestion of infected tissue. Diarrhea, which may become bloody, is the primary symptom. Most animals infected with coccidia are asymptomatic; however, young or immuno-compromised animals may suffer severe symptoms, including death. Among domestic animals, industrially bred poultry and rabbits are particularly prone to this disease. Today, 11 coccidiostatic agents are authorized as feed additives in accordance with EU regulation 2003/1831/EC. As feed companies typically use the same production line for the production of different feeds, carry-over and therefore transfer of coccidiostats from one batch to another is unavoidable. Maximum levels of coccidiostat carry-over have been set (2009/8/CE) and this directive sets maximum carry-over levels of 1% for sensitive animal species. Feeds are very complex and diverse mixtures to analyze in a routine environment. It is known that matrix components can significantly alter the response in electrospray ionisation, either by signal enhancement or suppression. Matrix effects can be minimised by reducing the absolute amount of matrix ions in the source region. One way that this can be achieved is to dilute the samples (if this method used is sensitive enough to permit this) and hence reduce the matrix loading on-column. This poster describes a fast, accurate and robust UPLC/MSMS method using the Waters ACQUITY UPLC I-Class coupled to a Xevo TQ-S for the detection of 11 coccidiostatic agents in feed at levels down to 0.25% carry-over levels.

Keywords: *Coccidiostats, LC-MS/MS, feed, veterinary, drugs*

S-23
DISCOVERY OF MULTIPLE SITES OF INTRA-MOLECULAR PROTONATION AND DIFFERENT FRAGMENTATION PATTERNS WITHIN THE FLUOROQUINOLONE CLASS OF ANTIBIOTICS IN PORCINE MUSCLE EXTRACTS USING ION MOBILITY MASS SPECTROMETRY

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In this study we report the use of High Definition Mass Spectrometry (HDMS) as a powerful tool for method development to support the unequivocal identification of residues of fluoroquinolone antibiotics in crude porcine tissue extract. High Definition mass spectrometry is a combination of high resolution mass spectrometry and high efficiency ion mobility based measurements and separations. Ion mobility spectrometry (IMS) is a rapid orthogonal gas separation phase technique which allows another dimension of separation to be obtained within an Ultra Performance Liquid Chromatography (UPLC) timeframe. Compounds can be differentiated based on their size, shape and charge. In addition, both precursor ion and fragment ion information can be simultaneously acquired in a single injection in an HDMS experiment, referred to as MSE. The unique advantages of HDMS as a technique for profiling complex matrices are also discussed. The enhanced analytical performance has facilitated the detection of antibiotics in a selection of sample types requiring only a simple and generic extract preparation step. HDMS can not only provide additional peak capacity but also insights into the molecular characteristics of the analytes during the method development process for example, the elucidation of multiple sites of protonation within a single compound. The additional information obtained has been used to inform and optimise the analytical strategy adopted. The ion mobility data generated has been used to calculate the collision cross section area (CSS) values for the target antibiotics. The combination of CCS values, retention time, exact mass and fragmentation information provides a unique and unequivocal characteristic signature of a compound. In addition, CCS values could serve as an invaluable identification point (IP) which could be utilised during the analyte confirmatory process in case of a non-compliant sample being identified.

Keywords: *Fluoroquinolones, ion mobility, collision cross section, QTof*

S-24
MULTICLASS DETERMINATION OF ANTIBIOTICS IN HONEY

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Antibiotic drugs are not authorized for the treatment of honey bees in the European Union; thus, there are no Maximum Residues Limits established. However no harmonization has been defined worldwide and in many third countries some antimicrobials are authorized for the treatment of honey bees. Moreover illicit treatments are well documented both in the EU and in third countries. For an example, almost all notifications of the Rapid Alarm System for Food and Feed (RASFF) in honey bee products involve the presence of antimicrobial residues. In the last five years (2008–2012) the most found compounds were sulfonamides (29%) followed by tetracyclines (18%) and macrolides (15%). The rest were aminoglycosides, chloramphenicol, lyncomicin, nitrofurans, nitroimidazoles and quinolones. Therefore if in the global market era the availability of suitable analytical methods is fundamental, the increasing use of multiclass procedures also in veterinary drugs field largely improves the cost-effectiveness of the analytical controls. For this purpose a multiclass approach for the confirmation of antimicrobial substances in honey has been developed and validated according to Commission Decision 2002/657/EC. A total of 27 basic drugs belonging to sulfonamide (10), nitroimidazole (9) and quinolone (8) families were determined. Sample preparation consisted in acidic hydrolysis of honey followed by a double purification step (defatting and strong cation exchange solid-phase extraction). The instrumental determination was achieved by liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC Surveyor coupled to TSQ Quantum Ultra, Thermo Scientific) operating in positive electrospray ionization mode. Chromatographic separation was performed on a Poroshell 120 EC-C18 column (100 × 3.0 mm, 2.7 µm) using a gradient with acetonitrile and water both containing 0.1% of formic acidu The method was validated in the range 0.1–10 µg/kg (0.1, 0.33, 1.0, 3.3 and 10 µg/kg) evaluating selectivity, linearity, precision, trueness, decision limits and detection capabilities. The procedure was applied to analysis of 50 honey samples of different botanical origins and geographical provenience collected from Italian market. In six honeys (12%) trace levels of sulfonamides were found. In three samples the presence of sulfathiazole was confirmed (0.3, 0.6 and 2 µg/kg), as well as that of sulfadimethoxine in three cases (0.2, 0.4 and 0.7 µg/kg). Sulfathiazole was detected only in honeys of Italian origin and sulfadimethoxine in samples also containing honeys of foreign origin (Argentine and Hungary). Considering the found levels there are no concerns about public health.

Keywords: *Veterinary drugs, honey, LC–MS/MS*

Acknowledgement: *The authors gratefully acknowledge financial support from the Italian Health Ministry (Ricerca Corrente IZSUM RC 02/11)*

S-25
RESIDUAL STUDY IN LIVER AND IN BILE AFTER A GROWTH-PROMOTING TREATMENT IN BULLS

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The use of anabolic steroids is prohibited in food producing animals in the European Union. Because efficient control must both consider metabolic patterns and associated kinetics of elimination, in this work a residual study in bovine liver and bile after a typical growth promoting treatment is described. Sixteen bulls were implanted with Revalor-XS® (trenbolone acetate and estradiol) for 70 days and slaughtered on the following day. At the same time sixteen bulls were kept as control group. A liquid chromatography–tandem mass spectrometry method (LC–MS/MS) was developed and validated to detect a selected package of steroids: androstenedione, dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), alpha-estradiol, beta-estradiol, estriol, estrone, alpha-testosterone, beta-testosterone, alpha-trenbolone and beta-trenbolone in liver and bile. Individual isotope-labeled analogues were used as internal standards. Sample preparation involved enzymatic hydrolysis and solid-phase clean-up. Prior to LC injection, the purified extracts were derivatized with hydroxyl amine to improve the ionization efficiency of the most analytes. The chromatographic separation was achieved using a Kinetex C18 column (100 × 2.1 mm, 2.6 µm). For the analyte detection two separate chromatographic ru ns were carried out: in positive electrospray ionization mode for all the derivatizable steroids and in negative mode applying APCI source for the others (estradiol and estriol). The presence of both isomers of the administered synthetic steroid trenbolone was detected in liver of all the treated animals. The measured levels ranged between 0.06 and 1.1 µg/kg for alpha-trenbolone and between 0.05 and 0.20 µg/kg for beta-trenbolone. Among the natural steroids investigated, only alpha-testosterone, beta-testosterone, DHEA, androstenedione and alpha-estradiol were found in hepatic tissue both in the treatment and in the control group. In bile the concentrations of the found analytes were generally higher than in liver. For an example, biliary concentrations of alpha- and beta-trenbolone in implanted bulls ranged from 15 to 93 µg/kg and from 0.08 to 0.77 µg/kg, respectively. The natural steroids found in bile were the same as in liver, but, in addition, in bile also estrone was present at detectable levels. Finally between the two groups some significant concentration differences were observed. Generally the levels of some estrogens increased in the tr eated animals, whereas those of some androgens decreased (Mann-Whitney test).

Keywords: *Bulls, growth-promoting treatment, liver, bile, LC–MS/MS*

Acknowledgement: *The authors gratefully acknowledge financial support from the Italian Health Ministry (Ricerca Finalizzata RF-IZV-2008-1175188 "Application of innovative techniques (OMICS) and classical analytical techniques for the detection of biomarkers and residues of illicit treatments of bovines with growth promoting agents") and Merck Animal Health for kindly supplying the implant Revalor-XS®*

S-26
ALTERNATIVE VALIDATION OF A METHOD FOR THE DETERMINATION OF RESIDUES OF ß-LACTAM ANTIBIOTICS IN MUSCLE BY LC–MS/MS

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The ß-lactam antibiotic groups penicillins and cephalosporins are widely used in the treatment of infectious diseases. Low-level doses consumed for long periods of time, e. g. in foodstuffs, can lead to the spreading of drug-resistant micro-organisms. Maximum residue limits (MRLs) in different food matrices were established by European Regulation (EC) No 470/2009 and subsequent modifications to ensure human food safety. The presented method was specifically developed for the determination and confirmation of the above-mentioned substance groups in muscle by LC–MS/MS in the ESI+ mode, and is based on a multi-method for different antibiotic groups in several matrices. The method was validated mainly in a concentration range of 0.25 to 2.0 MRL. The validation showed that the method was applicable to different muscle samples of cattle and pig. The validation was arranged in accordance with Commission Decision 2002/657/EC. Different influencing factors were selected with regard to the requirements of different samples and varying conditions in the laboratory. Therefore an efficient validation approach was necessary. The study was performed on the basis of a factor-comprehensive in-house validation concept applying an orthogonal experimental design and was realised with the help of InterVal. Using this concept the validation of the method was successfully accomplished with a limited number of experiments. The relevant validation parameters, e.g. the analytical limits (CC-alpha and CC-beta), are presented and discussed

Keywords: *Residue method, muscle, ß-lactam antibiotics, alternative validation, LC–MS/MS*

S-27
PRODUCTION OF POLYCLONAL ANTIBODIES FOR THE DETERMINATION OF FLUOROQUINOLONE RESIDUES IN MILK BY ENZYME-LINKED IMMUNOASSAY

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Fluoroquinolones (FQs) represent a recent and highly potent group of antibiotics used in human and in veterinary medicine. These compounds are broadly used in the treatment of respiratory disease and enteric bacterial infections in humans and in food-producing animals such as cattle, swine, chicken and so on. The widespread use of FQs in agriculture has resulted in the presence of these compounds residues in foodstuffs from animal origin. These residues are a source of concern due to the emergence of drug-resistant bacteria and they are also a potential health hazard for consumers. Therefore, the European Union and Russian Federation has set Maximum Residue Limits (MRLs) for FQs in animal products such as milk. There are five quinolones regulated in bovine milk. The MRL established for these quinolones ranges between 30-100 μg kg-1. These low values demand the development of screening methods that are sensitive enough to monitor and determine these drugs in bovine milk. In this study, ELISA method was developed and optimized to allow the detection of the FQs residues in bovine milk. A new adjuvant "Floravit" was used for the production of polyclonal antibodies against fluoroquinolones. "Floravit" is a bioactive additive based on cultural medium of Fusarium sambucinum. Three schemes of rabbit immunization were applied for anti-FQs antibodies production:
1) Immunogen was emulsified with complete Freund's adjuvant for primary immunization, booster immunizations were performed using saline;
2) Immunogen was emulsified with complete Freund's adjuvant solved in bioactive additive "Floravit" for primary immunization; booster immunizations were performed using the bioactive additive "Floravit";
3) Immunogen was emulsified with complete Freund's adjuvant solved in 10^{-6} dilution of "Floravit" in saline for primary immunization, booster immunizations were performed using 10^{-6} dilution of "Floravit" in saline. The antibody titer, the IC_{50} for different FQs and the antibodies specificity were assessed by indirect ELISA. The IC50s for ciprofloxacin and enrofloxacin were (26.0±16.0); (7.2±4.6); (4.5±0.9) μg L^{-1} for antibodies produced by the 1st, 2nd and 3rd scheme of immunization subsequently. Cross-reactivity with other FQs, except flumequin was more than 10%. The highest antibodies titer was observed for antibodies produced by 3rd scheme of immunization. It concluded that 10^{-6} dilution of "Floravit" in saline could be considered as a suitable for the production of polyclonal antibody to FQs. The antibodies produced by last scheme of immunization were applied for the determination of FQs in milk. The method was validated, in line with EU requirements (Commission Decision 2002/657/EC) concerning screening methods. Recoveries higher than 80% were obtained for all quinolones. The limit of quatification was 3 μg kg^{-1}.

Keywords: *Fluoroquinolone, residues, ELISA, polyclonal antibody, adjuvant*

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S-28
SURVEY OF VETERINARY DRUGS IN FOODS, KOREA

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This study has been conducted to determine the residual amount of Veterinary drugs such as Meloxicam, Flunixin, Tulathromycin stock farm products (beef, pork, horse and milk). Veterinary drugs have been widely used in the rearing of food producing animals to prevent and treat diseases. About 300 Samples were purchased from the markets located in the major cities (Seoul, Busan, Incheon, Daegu, Daejeon, Gwangju, and Ulsan) in Korea. Veterinary drugs were analyzed by LC–MS/MS according to the Korean Food Code. For the confirmation of detected antibiotics in sample, positive samples were analyzed by LC–MS/MS according to the Korean Food Code. All analytical procedures were validated in accordance with CODEX guidelines. The monitoring data were satisfied with criteria of CODEX, which located within 70~120% recovery range, less than 20% of relative standard deviations. This study result will provide useful information consecutive monitoring project to guarantee food safety.

Keywords: *Veterinary drugs*

S-29
A SCREENING/CONFIRMATORY METHOD FOR ß-AGONISTS IN URINE SAMPLES USING HPLC–MSMS

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The use of growth promoting agents such as β-agonists in food producing animals is banned in Uruguay, and other Latin American countries. The β-agonists are banned for alleged adverse effects on human health and their effect on growth rate and feed conversion in food producing animals. They enhance growth rates and improve the muscle/fat ratio with a better conversion of feed into meat instead of fat. Monitoring such compounds requires analytical techniques with acceptable sensitivity and selectivity levels. The use of immunoaffinity columns for sample clean-up improves such attributes. This paper outlines a multi-residue analytical method based on HPLC–MSMS and developed/validated according to European Union Decision 2002/657/EC, to determine ten β-agonists in bovine urine.

Keywords: *ß-agonists, multi-residue, urine, HPLC–MSMS*

Acknowledgement: *To the IAEA, for their support under the ARCAL RLA5059 Project*

S-30
TARGETED ACCURATE MASS DETECTION OF VETERINARY DRUGS AND OTHER CONTAMINANTS IN FEED BY UHPLC–HRMS

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Toxic substances such as veterinary drugs, mycotoxins, plant toxins and ergot alkaloids are frequently present in animal feed due to their misuse or also because of the environment. The monitoring and analysis of the presence of these potentially hazardous chemicals remains one of the main tasks to ensure feed safety. Thus, the trend to guarantee quality and safety of feedstuff is going towards targeted screening approaches using high resolution mass spectrometry (HRMS). In this work, an ultra high performance liquid chromatography-high resolution mass spectrometry (UHPLC–HRMS) methodology is proposed for the multi-class multi-residue screening of veterinary drugs and other contaminants such as undesirable substances regulated by the European Union in feed, using an Orbitrap Exactive™ analyzer working at a resolving power of 50.000 FWHM in full scan mode, both in positive and negative mode. A database including the elemental composition, the polarity of acquisition, retention time and expected adducts was built for the targeted analysis of around 70 substances. High mass accuracy minor than 5 ppm was set as one of the identification criteria. A “one step” sample clean-up procedure was developed based in the QuEChERS methodology allowing the extraction of a wide range of molecules. An efficient chromatography was achieved using ultra high performance liquid chromatography with Thermo Hypersil Gold 100x2.1 mm 1.9µm column with a run time of 15min. The rapid sample preparation procedure combined with the developed chromatographic method and the high resolution mass spectrometry detection makes it an ideal tool for compliance monitoring in regulatory laboratories. The suitability of full scan HRMS method for quantitative and confirmatory purposes on the basis of the Commission Decision 657/2002/CE was evaluated for each substance and results are presented and discussed.

Keywords: *Veterinary drugs, contaminants, feed, LC–HRMS, Orbitrap*

S-31
QUALITATIVE CONFIRMATORY METHOD FOR DETERMINATION OF PHENYLBUTAZONE AND 11 PROHIBITED NSAIDS IN ANIMAL MUSCLE AND MILK BY UPLC–MS/MS

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Phenylbutazone and other non-steroidal antiinflammatory drugs have been reported recently by the EC Rapid Alert System and the media, because of their presence in food producing animals. To carry out a more complete regulatory control programme for not allowed NSAIDs, a sensitive and quick qualitative confirmatory method was developed in our laboratory. Phenylbutazone, oxyphenbutazone, carboxyibuprofen, piroxicam, naproxen, niflumic acid, flurbiprofen, suxibuzone, indometacin, mefenamic acid, flufenamic acid and meclofenamic acid, were determined in animal muscle and milk. After a chemical hydrolysis in tissue and an organic extraction in milk, the extracts were cleaned up by a quick SPE (C8), which reduced fat components and ion suppression in order to increase sensitivity and reproducibility in both matrices. SPE eluates were evaporated, redissolved and injected in a UPLC–MS/MS system. Only 8 min of runtime were necessary per sample. Detection capabilities were lower or equal than the Recommended Concentrations (RCs) by the European Community Reference Laboratories (phenylbutazone, 2.5 µg/kg; oxyphenbutazone, 5 µg/kg; naproxen, 10 µg/kg; mefenamic acid, 5 µg/kg) and as low as possible for the substances for which no regulation has been set in milk and muscle (carboxyibuprofen, 5 µg/kg; piroxicam, 2.5 µg/kg, niflumic acid, 2.5 µg/kg; flurbiprofen, 20 µg/kg; suxibuzone, 5 µg/kg; indometacin, 5 µg/kg; flufenamic acid, 5 µg/kg; and meclofenamic acid, 5 µg/kg).

Keywords: *Phenylbutazone, NSAIDs, animal muscle, milk, UPLC-MS/MS*

S-32
PERSISTENCE OF METRONIDAZOLE RESIDUES IN BLACK TIGER SHRIMP (*PENAEUS MONODON*)

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Metronidazole (MNZ) is an imidazole heterocycle substituted with a nitro functional group on the fifth position on its ring. It can be used for treating parasitic infections in farmed aquaculture species. MNZ is metabolised in vitro through oxidation of the side chain in the C-2 position of the imidazole ring to form MNZ-OH. Both MNZ and MNZ-OH are suspected to be carcinogenic and mutagenic to humans. As a consequence their use in food-producing species is prohibited within the European Union. In order to protect public health and harmonise trade, the Berlin Community Reference Laboratory has assigned a recommended level of 3 µg kg^{-1} to selected 5-nitroimidazole residues and their hydroxy metabolites. The study of the effect of metronidazole contamination in black tiger shrimp (Penaeus monodon) was carried out as follows. Shrimps were immersed in a 390L tank containing 50 mg MNZ per L for 24 h (n=4 tanks). Following treatment, shrimps were transferred to sea water and samples were collected immediately (day 0), day 1, day 2, day 4 and day 8. A total of 12 shrimps were analysed per time point using UHPLC–MS/MS method in positive ionisation mode. MNZ and MNZ-OH were detected in shrimp tissue samples at concentrations of 361–2631 and 0.55–6.55 µg kg^{-1} on the day 0, respectively. Residue concentrations rapidly depleted in tissue samples at later time points but MNZ residues were still measureable at 0.14–0.70 µg kg^{-1} on day 8. The results of the depletion study demonstrate rapid elimination of metronidazole residues in prawn. MNZ is the most persistent marker residue and can be detected for at least 8 days post-treatment. Whereas MNZ-OH residues were only detectable at day 0.

Keywords: *Nitroimidazole residues, metronidazole, metronidazole-OH, UHPLC–MS/MS, shrimp*

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S-33
RESIDUES OF AVERMECTINS IN THE THERMO-PROCESSED MEAT PRODUCTS EXPORTED FROM BRAZIL

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The National Plan for Control of Residues and Contaminants in products of animal origin (PNCRC/Animal) is an official program under responsability of Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) which seeks to evaluate and maximize the guarantees of chemical safety provided from the Brazilian production systems and their products of animal origin. Some of these granted chemicals monitored are the Avermectins, a group of antiparasitary substances which is under official monitoring in cattle production system since 2000, in the cattle liver. Even considering that liver is the target matrix for this class of substances, as recommended by the Codex Alimentarius, but also considering some international notification received from other countries regarding non-compliances of residues of Avermectins in thermo-processed meat products exported from Brazil, from 2010 on the PNCRC also included the cattle muscle in an exploratory subprogram. The q uantification method used to perform these analyzes was the High-Performance Liquid Chromatography with Fluorescence Detector (HPLC–FLD) and the confirmatory method was the High-Performance Liquid Chromatography coupled to Mass Spectrometers (HPLC-MS/MS). From the year of 2010 to 2013 were analyzed 1722 samples, which 652 samples were performed in cattle liver and 1070 samples in cattle muscle, being detected 15 violations of Avermectins: 13 violations in liver samples and only 02 in muscle. The PNCRC results indicated some foreseen findings: a very low general prevalence (only 0.87%) of violations of Avermectins in cattle and a larger number of violations found in liver (1.99%) than in cattle muscle (0.19%). Therefore, this official result clearly confronts the international notification of non-compliances of residues of Avermectins in thermo-processed meat products exported from Brazil. This significant difference may indicate some interferences of other factors in the moni toring of importing countries, mainly because: these substances are metabolized in the liver, not in muscle; there's no international setting nor recommended limits for thermo-processed meat products; in fact, should be considered the possible concentration effect due the muscle groups normally used for these kind of meat products, which are the same common site of application of Avermectins; in other hand, should be considered a dilution effect that normally occurs during the processing of these kind of meat products, being unlikely to occur a huge number of violations in thermo-processed meat products. These results indicate the needing for specific studies to evaluate the factors influencing the concentration of avermectin during the processing of meat products, providing information to support the setting of appropriated limits and the adequacy of the analytical methodology adopted by any country, contributing for the improvement of chemical safety provided by the ther mo-processed meat products exported from Brazil.

Keywords: *Avermectins, Residues, Thermo-Processed Meat Products*

Acknowledgement: *Ministry of Agriculture of Brazil*

S-34
HIGH RESOLUTION MASS SPECTROMETRY FOR THE QUANTITATIVE ANALYSIS OF TARGETED RESIDUES IN FOOD SAMPLES: APPLICATION TO VETERINARY DRUG RESIDUES AND QUALITY ASSURANCE ISSUES

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Current trends in chemical residue analysis open the field toward high resolution mass spectrometry (HRMS) technologies. HRMS is reported to be a powerful tool for the reliable identification of analytes in complex matrices. Further to the currently typical methodologies using liquid chromatography coupled to quadrupole tandem mass spectrometry (LC–MS/MS), HRMS analysis requires more powerful analyzers (e.g. Time of Flight, Fourier Transform Orbital Trap) thanks to their high resolution power, mass accuracy and sensitivity. Full scan MS acquisition coupled to the Fourier Transform Orbital Trap Measurment (Orbitrap®) becomes a helpful tool as well for the detection and identification of targeted analytes as for non targeted ones at low µg/kg level of concentration. This approach showed to be an effective means for the qualitative and semi quantitative screening of various veterinary drugs residues in food. Less is known about HRMS analytical performances and limitations regarding the quantitative determination of target residues. In this respect, an analytical method for the rapid and reliable quantification of ceftiofur residues (i.e. a third generation cephalosporin antibiotic, C3G) in poultry tissues is presented. The developed methodology consists in the hydrolysis and subsequent derivatization of ceftiofur in samples, then an extraction from biological samples step onto Sep Pack C18 cartridges, and a chromatographic separation (LC) followed by a full scan accurate mass data acquisition under electrospray positive ionization mode at a resolution of 60,000 FWHM. The largest m/z range as possible was chosen (i.e. 100–1000 Da) in order to preserve the opportunity for the "a posteriori" screening (i.e. after FT MS acquisition) of other residues potentially characterizing the exposure to ceftiofur. Particular care will be taken to highlight the performances and limitations of the whole method through the description of and discussion about its validation performance parameters, i.e. repeatability, reproductibility, specificity, limit of detection, but also its limit of quantifications, with respect to the use of isotope-labeled internal standard, for the analysis of non-authorized ceftiofur in chicken tissues. Therefore, critical considerations about prescribed quality assurance issues extracted from the European regulatory decision 2002/657/EC and the continuous flowering progress of analytical technologies will be pointed out through the herein presented method. Results obtained from the analysis of ceftiofur performed on treated and non-treated chicken samples will also illustrate the options HRMS methodology may offer.

Keywords: *Veterinary drugs, High resolution mass spectrometry, Orbitrap, Quantitative analysis*

S-35
THE USE OF MICROFLOW UHPLC IN VETERINARY DRUG RESIDUE ANALYSIS

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Traditionally in veterinary drug residue screening of food samples, samples are extracted and analysed by LC–MS/MS usually at LC flow rates which are in excess of 500 µl/min and in combination with high pressures with smaller particle size HPLC columns to maintain sharp peaks and fast chromatography. These flow rates produce fast speeds and excellent peak shapes and results, but have a draw back in that they require higher volumes of organic solvent. The consumption of HPLC organic solvents, such as acetonitrile and methanol, is a growing cost of analysis and its disposal has an environmental impact. Therefore, ways to reduce solvent consumption in pesticide residue testing will be beneficial to the environment and reduce running costs of a testing lab. Here we present new data using microflow LC, running below 40 µL/min, in combination with a LC–MS/MS method developed on an AB SCIEX QTRAP® system utilizing the Scheduled MRM™ algorithm. Initially this approach has been applied to a screen of veterinary residues including sulphonamides to show its applicability in food analysis and data presented with compare Micro LC with traditional LC flow rates.

Keywords: *LC–MS/MS, Micro LC, Veterinary Residues*

S-36
RAPID DETERMINATION OF 25 SULFONAMIDES AND THEIR METABOLITES IN ANIMAL TISSUES BY HPLC – HIGH RESOLUTION ORBITRAP MASS SPECTROMETRY

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Currently, veterinary drugs are recognized not as only medicines but also as 'newly emerging contaminants' in the environment. Pharmaceutical residues such as antibiotics and, in particular, sulfonamides (SAs) represent an essential part of these emerging contaminants. After tetracyclines, SAs are the most commonly used veterinary antibiotics in the EU due to their low cost and relative efficiency in combating many common bacterial infections. As a consequence of their extensive usage, unwanted residues of SAs including parent compounds, metabolites or degradation products, can persist in many biological matrices (edible animal tissue, milk…), presenting a risk to consumers. Because of their possible side effects, the usage of SAs in animals is regulated by law in many countries. According to the European Union (EU) regulations N0 37/2010, the maximum residue limits (MRL) for the total amount of SAs in biological matrices used for human consumption, such as muscle, liver, kidneys and milk is 100 µg/kg [1]. Therefore, the monitoring of these compounds in animal tissue has become a priority.
Here, we present the development of a sensitive method for a rapid determination of 25 SAs and their metabolites in animal tissue by QuEChERS-based extraction and LC–ESI LTQ Orbitrap MS. The performance of the method has been evaluated in accordance with the EU guidelines, and the method was validated with a reference material. Finally, the method was successfully applied to various matrices (kidney, liver, muscle) originated from real samples (pig, chicken, beef and sheep,), collected from Lebanon and France, allowing the simultaneous screening of target sulfonamides and identification of untargeted compounds such as N^4-Acetyl metabolites.

[1] COMMISSION REGULATION (EU) No 37/2010 of 22/12/2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Journal officiel de l'Union européenne, 2010. 15: p. 1-72

Keywords: *Sulfonamides, metabolites, Orbitrap, Quechers*

Acknowledgement: *Lebanese National Council for Scientific Research (CNRSL), Lebanese Atomic Energy Commission (LAEC)*

S-37
SIMPLE AND HIGH THROUGHPUT DETERMINATION OF NITROIMIDAZOLE RESIDUES IN MUSCLE BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Nitroimidazoles are synthetic antimicrobial agents used against bacterial and coccidian infections in poultry, swine and cattle. Because of the mutagenic and carcinogenic properties of the nitroimidazole moiety, dimetridazole, metronidazole and ronidazole are included in the list of prohibited substances of Regulation (EU) No 37/2010. Moreover, no other compounds of this family have an assigned MRL. Thus, in practice, the zero tolerance principle applies to the whole group. Liquid chromatography coupled to triple quadrupole mass spectrometry, after an extraction and a clean-up by solid phase extraction (SPE), is the technique commonly applied in official control laboratories. The purpose of the present work was the development and validation of a high throughput method for the analysis of up to 11 nitroimidazoles, including some metabolites. In preliminary experiments acetonitrile (ACN) and ethyl acetate were compared as extraction solvents. Because ACN provided higher recoveries and cleaner extracts, it was selected for the further work. In this scenario, the QuEChERS methodology, based on the use of ACN was assessed. In the extraction step, the influence of the amount of NaCl and $MgSO_4$ was assessed, while C18 and PSA were studied in the dispersive SPE step. The highest recoveries were obtained without using salts in the extraction stage and omitting the dispersive cleanup. After evaporation to dryness, ACN extracts were reconstituted with water, filtered and injected in the chromatographic system. The high polarity of nitroimidazoles enables them to dissolve in water while most of the co-extracted matrix components remain undissolved. This leads to clean extracts that show negligible matrix effects. In these conditions, the recoveries obtained from spiked muscle samples of swine, lamb, chicken and rabbit were very similar and fell in the range 83–97%, except for ipronidazole (42%) and dimetridazole (68%). The recoveries obtained from cattle muscle were a little bit lower for most of the compounds. The method was validated according to the requirements of Decision 657/2002/EC. Calibration curves in the range 0.5–10 ng/g were prepared from surrogate matrix matched standards. Accuracy values were in the range 90–106%. Reproducibilities, expressed as RSD%, lower than 10% were obtained. The values of CCα (0.05–04 ng/g) and CCβ (0.1–0.5 ng/g) point out that the method is suitable for an "as low as possible" approach. The method also provided satisfactory results when it was applied to retina, kidney and liver tissues.

Keywords: *Nitroimidazoles, residue analysis, liquid chromatography, mass spectrometry*

S-38
DETERMINATION OF AVERMECTINS AND MILBEMYCINS RESIDUES BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY: TRIPLE QUADRUPOLE VERSUS QUADRUPOLE-ORBITRAP INSTRUMENTATION

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Macrocyclic lactones such as avermectins and milbemycins are anti-parasitic agents widely used in the treatment of food producing animals, and maximum residue levels in specific edible matrices have been established by the European Union. Currently, liquid chromatography coupled to triple quadrupole mass spectrometer (LC–QqQ–MS/MS) is the technique of choice in confirmatory residue analysis. However, it has been reported that most macrocyclic lactones show low ionization efficiency. In addition, when using positive electrospray (ESI) they tend to form sodium adducts, that provide low linearity and poor fragmentation. Negative electrospray is an alternative, although it offers less sensitivity. This study focuses on the development of a simple confirmatory method, based on LC–QqQ–MS/MS, for the analysis of ivermectin, abamectin, emamectin, doramectin, eprimectin and moxidectin in animal tissues. In addition, the performance of a Q-orbitrap mass spectrometer has been also investigated. A QuEChERS approach has been used for sample treatment. Thus, the analytes are extracted from the minced tissue sample with acetonitrile in the presence of sodium chloride and magnesium sulphate and the organic extract is cleaned-up by dispersive solid phase extraction with C18. Extraction recoveries for the analytes range from 74% to 101%. The analytes are determined by LC–QqQ–MS/MS. The chromatographic separation is performed at 40°C in gradient elution mode using a Luna C18 column and acetonitrile/triethylamine aqueous solution mixtures as mobile phase. Negative ESI conditions and multiple reaction monitoring are used. For all the analytes [M-H]- is the precursor ion and two fragment ions are recorded. The high-throughput QuEChERS LC–MS/MS method, has been validated in muscle matrix, according to Decision 657/2002/CE guidelines. On the other hand the capabilities of the Q-Orbitrap high resolution mass spectrometer have been explored. Data dependent (ddpp) acquisition modes enables to focus on the most intense ions among those that have been previously defined as target ions, and provide an excellent performance either with positive or negative ionization, being the positive the most sensitive. For instance, using positive ionization and the ddpp t-SIM mode all the analytes can be confirmed at 0.5 μg kg^{-1} in muscle tissues. HRMS is a very powerful tool, which complements the use of QqQ instruments, for the control of residues in samples from animal species, when no MRL has been established and the lowest achievable limits have to be met.

Keywords: *Residue analysis, low resolution mass spectrometry, high resolution mass spectrometry*

S-39
GC–C–IRMS FOR ANALYSIS OF NATURAL HORMONE ABUSE IN CATTLE

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The use of hormones as growth promoters for fattening purposes in cattle has been banned in the European Union since the early eighties. Control of the illegal use of natural steroid hormones in cattle is still a challenge since no conclusive method and non-ambiguous analytical criteria are available. The ability of gas chromatography / combustion / isotope ratio mass spectrometry (GC–C–RMS) to confirm the administration of estradiol and testosterone to cows has been investigated. This was done by comparison of the $^{13}C/^{12}C$ isotopic ratio of the main urinary metabolites, i.e. 17α-estradiol and 17α-testosterone, with endogenous reference compounds (ERCs) to differentiate the endogenous or exogenous origin. For this purpose sample clean-up methods are developed based on humane urine analysis protocols which are used to determine if an athlete has been tested positive by administration of exogenous natural hormone. Preliminary results of the developed clean-up method and GC–C–IRMS analysis are presented.

Keywords: *GC–C–IRMS, natural hormones*

S-40
EFFECT OF CLENBUTEROL ON THE STERIODOGENESIS

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Clenbuterol is a well-known growth promotor in cattle. It has been used for decades reaching its peak use in Europe in 1995. Due to better detection strategies and therefore an enlarged change that illegal treated animals will be detected by control agencies, the number of positive findings has decreased drastically over the last 10 years. In countries outside Europe, however the opposite is observed. It has been found that people traveling to China were tested positive for clenbuterol in urine by eating clenbuterol contaminated meat. This contamination also occurs in other countries. Last year, in Mexico almost all soccer players competing in the FIFA U-17 World Cup 2011 were tested positive for clenbuterol. Again, this finding proves that clenbuterol is used on a large scale and that clenbuterol can cause positive doping test results or (in the worst case) poisoning of humans. In a recent animal trial conducted by the Technical University of Munich whereby calves were treated with anabolic steroids and clenbuterol, it was demonstrated that animals treated with clenbuterol gain an average 25 kg in weight more than non-treated animals. All samples from this study were used to assess the effect on the steroidogenesis of cattle by determining whether treatment has effect on the circulating endogenous hormones. The animal experiment consisted of 7 control animals and 7 animals treated with clenbuterol. Samples of urine were taken before treatment and 9, 23 and 34 days after treatment start. The samples were analysed using a rapid SPE clean-up which consists of an Oasis HLB 96-wells plate and a WAX 96-wells plate. Applying this method, most free compounds and their corresponding glucuronide and sulphate conjugates (total of 84 compounds) involved in the steroidogenesis are separated and analysed on a UPLC–MS/MS system within 6 minutes. After quantitative analysis of the urine samples, multivariate statistics was performed to determine which compounds are up- or down-regulated after treatment. First, the treated and non-treated groups of animals were trimmed using PCA. Subsequently, the groups were successful separated using OPLS–DA. Validation of the model was performed using cross validation and a permutation test. The compounds contributing to the separation of the groups were iden tified by means of an S-Plot. To our surprise it was found that the estrogenic compounds were affected by treatment with clenbuterol through down-regulation, in particular estrone-glucuronide and estradiol-glucuronide. The mechanism behind this effect is still not totally unravelled. Results of this study will be presented and discussed.

Keywords: *Clenbuterol growth promotor UPLC–MS/MS*

S-41
METABOLIC STUDY OF ENROFLOXACIN IN CHICKEN TISSUES BY LTQ–ORBITRAP AND MULTIPLE DATA PROCESSING TECHNIQUES

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Quinolones are one of the most widely used class of antibiotics in human and veterinary medicine. Their main uses in veterinary are therapeutic (treatment of bacterial infections), prophylactic (prevention of infections) and as growth promoters of animals intended for human consumption, although this last use is not allowed in the European Community. Misuse of antibiotics in animals and the medicated animal slaughter before the metabolism and excretion of the antibiotic after treatment, can lead to the presence and accumulation of residues of these antibiotics and their metabolites in tissues for human consumption. The presence of these residues can lead to health risks, such as allergy problems, toxicity and potential development of resistant bacterial strains. To avoid risks to consumer health, the European Community has established maximum residue limits (MRLs) for antibiotics according to each species and tissue. The regulation 37/2010 reflects the MRLs for permitted drugs. This regulation covers only the active compound and some metabolites, but other unknown metabolites could be present in tissues, which may become more harmful than the parent compound. In addition, some new transformation products (TPs) could be formed due to the complex sample treatment. The study included the analysis of samples from different animal tissues (muscle, liver and kidney) of chickens subjected to different days of pharmacological treatment with Enrofloxacin (ENR) with the aim to identify new metabolites and TPs. The therapeutic treatment involved a daily dose of 10 mg/kg during 4 days. Three types of samples were analyzed from the different tissues; those corresponding to the animal slaughter on the second and fourth day of the pharmacological treatment and those corresponding to the animal slaughter four days after treatment ends. In order to identify these new metabolites and TPs, combination of accurate mass spectral data from LTQ–Orbitrap and multiple data processing techniques (Multiple Mass Defect Filter, EIC's, mass spectra comparison, etc.) were performed. The confirmation and structural elucidation of these new metabolites and TPs was carried out by MS/MS experiments. As a result, several metabolites and TPs were identified from the different analyzed samples.

[1] Commission Regulation 37/2010. Off. J. Eur. Commun. 1 (2010)
[2] M.P. Hermo, J. Saurina, J. Barbosa, D. Barrón. Anal. Chim. Acta, submitted for the publication, 2013.
[3] F.J. Morales-Gutiérrez, M.P. Hermo, J. Barbosa, D. Barrón. J. Mass Spectrom., submitted for the publication, 2013.

Keywords: *Quinolones, chicken tissues, metabolites, high-resolution mass spectrometry, data processing*

S-42
A SENSITIVE ELISA FOR THE DETECTION OF DAPSONE IN SHRIMPS, EGG AND MILK

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Dapsone (4,4'-diaminodiphenylsulfone) is a synthetic sulfone active against a wide range of bacteria, but it is mainly used to treat Mycobacterium leprae infections (leprosy) and the skin condition known as dermatitis herpetiformis. In addition, it has been used in malaria prophylaxis and in the treatment of various dermatoses. Besides a medication in humans, dapsone is also used as an antibiotic in animals to prevent and treat diseases such as streptococcal mastitis and coccidiosis of cattle. The mechanism of action of dapsone is probably similar to that of the sulfonamides, which involves inhibition of folic acid synthesis in susceptible organisms. As with the sulfonamides, antibacterial activity is inhibited by p-aminobenzoic acid. Dapsone is listed in table 2 of Regulation (EC) no. 37/2010). The decision can be found in Regulation (EC) no. 3426/93. Veterinary medicinal products in this table are banned for use in/treatment of food-producing animals. In the EU, a technical guide has been published by the Community Reference Laboratories. In this guide a recommended concentration of 5 ng/ml (ppb) has been published for dapsone in muscle, meat and milk. Using polyclonal rabbit antibodies, a competitive ELISA was developed for the detection of dapsone in shrimps, egg and milk. Validation of this dapsone ELISA was performed according to the European decision 2002/657/EC. Objective of this validation study was to determine the detection capability (CCβ), limit of detection (LOD), cross-reactivity (specificity), precision (inter- and intra-assay variation), recovery, and stability of the ELISA. Obtained CCβ values: 0.1 ng/g for shrimps, 0.1 ng/g for egg and 0.1 ng/ml for milk. Obtained LOD values: 0.05 ng/g for shrimps, 0.1 ng/g for egg and 0.01 ng/ml for milk. Cross-reactivities in buffer: 100% with dapsone, 8.7% with sulfaquinoxaline,

Keywords: *Dapsone, veterinary drugs, residues, sulfone, ELISA*

S-43
MASS SPECTROMETRY WITH THE MATRIX-ASSISTED LASER DESORPTION / IONIZATION IN IDENTIFICATION OF RESIDUAL QUANTITIES OF ANTIBIOTICS IN FOODSTUFF

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The study of complex organic compounds takes a special place in analytical chemistry. Among such compounds include biopolymers, antibiotics, etc. Determination of antibiotics in foodstuffs - an important problem of analytical control. In this report the possibility of using mass spectrometry with matrix-assisted laser dissociation / ionization combined with time-of-flight mass analyzer (MALDI–TOF) for identifying macrolide antibiotics, in foods is considered. MALDI–TOF mass spectrometer Autoflex III smartbeam (Bruker) was used. To increase the resolution used in reflectron mode, the main parameters of the analysis: used ultraviolet nitrogen laser with a wavelength of 337 nm, a pulse length of 3 ns and power of the laser radiation in the range 106–107 W/cm^2. The mass range for scanning: 600–4000 Da. The spectra of the individual antibiotics using α-cyano-4-hydroxycinnamic, 2,5-dihydroxybenzoic acid and sinapic acid as matrices are received. Preferable application of α-cyano-4-hydroxycinnamic acid as matrix is established. When using this matrix for recording the spectra of positive ions mixture of 8 antibiotics: monensin, erythromycin, narasin, josamycin, spiramycin, tilmicosin, tylosin, avilamycin ion peaks observed sequence of all antibiotics in the concentration range from 50 ng / ml. In the study of acetonitrile extract of milk showed signs of erythromycin, narasin and avilamycin. In the study of acetonitrile extracts of pork and beef were found narasin, monensin, erythromycin and avilamycin.

Keywords: *Matrix-assisted laser dissociation / ionization, mass spectrometry, antibiotics, macrolides*

S-44
DEVELOPMENT AND VALIDATION OF SCREENING AND QUANTIFICATION METHODS FOR MULTI ANTIBIOTIC RESIDUE DETECTION USING HIGH RESOLUTION MASS SPECTROMETRY

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Veterinary drugs are widely used for animal breeding to prevent or treat diseases or to promote growth. Abuse of veterinary drugs and its residues in food becomes an increasing problem due to the risk to human health, because they may lead to the appearance of drug-resistant bacteria. In the EU the use of veterinary drugs is regulated by Commission Regulation (EU) 37/2010 establishing Maximum Residue Limits (MRLs) and listing prohibited substances. All analytical methods have to be extensively validated in accordance to the requirements of the Commission Decision (EU) No 2002/657/EC with regard to the MRLs. An application of high resolution mass spectrometry for the detection of veterinary drugs in meat will be presented. The screening and quantification methods were developed and validated for the simultaneous determination of about 100 veterinary drug residues (benzimidazoles, quinolones, nitromidazoles, macrolides, triphenylmethan dyes, sulfonamides, tetracyclines, beta-lactams) in routine analysis. The quick and easy sample preparation based on liquid extraction with EDTA-succinate buffer and acetonitrile, followed by phase separation and evaporation of the supernatant. The extracts were solved in 0.1% formic acid and injected into an Accela™ LC systems coupled with a Thermo Scientific Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer. The analytes were detected by full-scan and data-dependent precursor ion fragmentation and interpreted applying TraceFinder™ software. The confirmation criteria were exact precursor mass, isotopic pattern, fragment ions, retenti on time and search against own spectral library containing MS/MS spectra of relevant analytes. The compound optimization included selecting precursor and fragment ions, optimization of collision energy, injection time and isolation window. For the quantification an external calibration curve was used and resulting concentrations were corrected with an internal standard for each analyte group and the recovery rate. Linearity of the instrument was tested by measuring of standards in solvent and the correlation coefficient for all components was higher than 0.995. The extraction recoveries were calculated about 70% on average. Signal suppression and enhancement were determined using matrix spiked with standards after extraction and found to be ≤30%. Both screening and quantification methods were successfully applied to real known and proficiency test samples including egg, fish and different meat matrices containing target analytes at very low concentrations. Therefore, the LC–HRMS approach could be a fast and an effective alternative not only for screening purposes, furthermore also in terms of quantification and confirmation.

Keywords: *HRMS, veterinary drugs, multimethod, screening*

S-45
IMMUNOCHROMATOGRAPHIC TESTS FOR VETERINARY DRUGS CONTROL IN FOODSTUFFS: SOLUTIONS TO INCREASE THE INFORMATION VALUE

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Immunochromatography provides special opportunities for screening of agricultural products and foodstuffs to possibility of "out of lab" testing, low labour intensity and rapidity. However, conventional tests control only exceeding a fixed threshold level by one target compound. Due to this methodological solutions that increase the information content of the testing are extremely useful. The tasks of primary interest are semi-quantitative assessment of the contamination and simultaneous control of several priority compounds. We have developed and characterized the corresponding tests for veterinary drugs of different classes (beta-lactams, fluoroquinolones, streptomycin, chloramphenicol) intended to monitor meat and dairy products. The possibility of directed shifting the immunoassay threshold via changes in the composition and concentrations of the reagents was studied. Multiparametric variations of gold particles size, composition of (colloidal gold - antibodies) and (antibiotic - protein) conjugates were carried out. It was shown that the threshold may be changed in 10–50 times (depending on target antigen and antibody affinity) without lost of assay accuracy. This allows to realize tests of antibiotics in different matrices, as well as multi-threshold semiquantitative assays using the same immunoreagents. Video digital registration of the bound label coloration was characterized as an additional tool to quantify subthreshold concentrations. Various interpolation methods of calibration dependences were compared. The possibility to determine the antibiotics content with an average deviation

Keywords: *Immunochromatography, test strips, antibiotics, multiplex assay, threshold value*

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S-46
ENANTIOMERIC SEPARATION OF CLENBUTEROL AS ANALYTICAL STRATEGY TO DISTINGUISH ABUSE FROM MEAT CONTAMINATION

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Clenbuterol is a beta-agonist which is therapeutically used as a racemic mixture for the treatment of reversible airway obstructions. In some countries Clenbuterol is approved as tocolytic in bovine and sheep. Clenbuterol is also proven to be abused in animal husbandry and sports for growth promoting purposes due to its anabolic properties. Lately adverse analytical findings in athletes were claimed to be due to contaminated meat. Already in 1995 Hemmersbach et al. presented an administration trial of Clenbuterol via meat of orally treated cattle resulting in urinary concentration in the sub-ng/mL range in the volunteers. In 2000 Smith et al. reported that the R(-)-Clenbuterol isomer is enriched in pork meat due to differences in pharmacokinetics in livestock. Ingestion of this different ratio from meat may lead to a different ratio in athletes urines with the perspective of a discrimination of the consumption via contaminated meat from illegal administration of Clenbuterol from drugs. In this context a method for the determination of the enantiomeric ratio of Clenbuterol in urine and meat, especially suitable for very low concentration levels, was developed. Enantioseperation was performed on a chiral column using high performance liquid chromatography (HPLC). The method development and validation are discussed. Also results of urine samples (human) collected after an administration study with Clenbuterol in yoghurt and results from urine samples from people having travelled to China and being exposed to Clenbuterol (possibly) via meat are presented. Results from the analyses of meat samples from a controlled animal trial are also discussed.

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[2] Smith. Et all, Stereochemical composition of clenbuterol residues in edible tissues of swine. J. Agric. Food Chem. 2000, 48, 6036.

Keywords: *Chiral separation clenbuterol doping lcmsms*

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S-47
A NEW, PRECISE AND ACCURATE METHODOLOGY FOR RAPID DETERMINATION OF 10 SULFONAMIDE RESIDUES IN MEAT SAMPLES IN ONE RUN BY USING LC–MS/MS

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Background. Sulfonamides are a group of synthetic anti-microbiological agents. These agents are used animal feeds, not only for bacterial infections, but also frequently illegally as additives for animal growth-promotion and they generate potentially serious problems for the human health, such as fatal allergic or toxic reactions. Furthermore, the main risk from the excessive use of anti-microbiological agents in animal feeds is, that bacteria may develop resistance. Additionally, some sulfonamides have been found to be potentially carcinogenic. These facts have become a cause for considerable debates in food safety. The European Union, Canada and USA regulations have set the MRLs (Maximum Residue Limits) of total sulfonamides of 100 µg kg^{-1} (ppb) in edible tissues. The AOAC (Association of Official Analytical Chemists) Official Method 983.31 Sulfonamide Residues in Animal Tissues and the US Department of Agriculture, Food Safety an Inspection Service, Method Nr. CLG-SUL2.06 Automated Robotic Extraction / TLC Analysis of Sulfonamide Residues in Animal Tissues, Eggs, and Egg Products are the present reference methods. These two methods are Thin-Layer Chromatographic Screening by fluorescence Densitometer Methods which take more than 2 hours for sample preparation time, and approx. another 2 hours for chromatographic conditions. The methods are applicable for the analysis of above mentioned sulfonamides in Muscle and Liver tissues of porcine, bovine, and avian species, and processed products at levels ≥ 0.05 mg/Kg.
Aim of the study. To develop an easy and fast method with very high sensitivity such as 2–26 ppt (0.002 to 0.026 µg/kg in solutions) for the determination of Sulfonamide Residues in Animal Tissues
Materials and methods. We developed an ultra-rapid and precise determination for 10 different Sulfonamide residues in meat samples by using LC–MS/MS: Sulfathiazole, Sulfaquinoxaline, Sulfapyridine, Sulfamethoxazole, Sulfamethazine, Sulfameter-ISTD, Sulfamerazine, Sulfadoxin, Sulfadimethoxin, Sulfadiazin and Sulfachloropyridazine). Animal meats bought from the local markets, LC–MS/MS based analysis performed with Agilent's Triple Quad MS 6460. Our Sample preparation is very easy and takes only 20 minutes compared to reference AOAC method with 2 hours. Weigh 5.0 g of minced cattle meat sample into a 50 ml polypropylene falcon tube, add 100 µl internal standard and 15 ml Reactive 1 onto sample and vortex 10 minutes. Centrifuge at 2000 rpm for 5 minutes. Transfer 1.0 ml from supernatant to a centrifuge tube and evaporate to dryness under stream of N_2 Add 3.0 ml Reactive 2 and 1.0 ml Reactive 3 onto residue and vortex. Take from the bottom layer with syringe and filter into HP LC vial and inject into the system.
Results. Jasem Sulfonamide method is very fast and with easy sample preparation of only 20 minutes (reference method at least 2 hours). For this reason this method has much lower cost of the sample preparation.

Keywords: *Food Analysis, Sulfonamide Residues, Meat, LC–MS/MS*

S-48
ANALYSIS OF VETERINARY DRUGS USING LC–MS/HRMS IN AN OFFICIAL FOOD SAFETY LABORATORY

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Veterinary drug residues are introduced into the food chain due to intensive farming practices, which have increased greatly since the mid 20th century. Food safety authorities around the world control their presence in food due to the health hazards they pose for humans. Joint FAO/WHO Expert Committee on Food Additives (JECFA), in accordance with standards laid by Codex Alimentarius Commission (CAC), evaluates veterinary drugs and establishes Codex maximum residue limits (MRLs). In the EU, the MRLs for veterinary drugs in different sample matrices are set out in the Commission Regulation No. 37/2010. Official control laboratories are an essential part of the food safety system, which monitor the drug residues in food by regular sampling and testing programs. In residue analysis, low resolution triple quadrupole mass spectrometer is widely used as confirmatory technique due to its robustness and ease to use. However, because of the low resolution of these i nstruments, it is hard to resolve isobaric matrix interferences, especially when residues of various chemical families or very complex matrices have to be monitored in the same analytical method. Moreover, apart from veterinary drug residues, there is possibility of presence of other contaminants such as pesticides, food contact materials, natural toxins, etc in the same sample. This inherently demands the change in testing methods from providing binary (residue present/ not present) results to comprehensive results (sample safe/ unsafe). This can be attained by comprehensive analysis of both targeted and non targeted analytes in the samples preferably at low concentration levels. Recently, high resolution mass spectrometry has evolved rapidly and offer impressive performance characteristics opening up new possibilities in food safety testing. In this presentation, different working modes of a quadrupole – Orbitrap hybrid mass spectrometer coupled to liquid chromatograph y (LC–MS/HRMS) and its implementation for routine testing in the Laboratory of the Public Health Agency of Barcelona are discussed. Moreover, some analytical case experiences with different chemical families of veterinary drugs (chromatographic analysis of aminoglycosides with HILIC/MS–HRMS, false positive case with analysis of ronidazole residue due to isobaric matrix interferences), and a non target workflow based on database approach will be covered.

Keywords: *Food safety control, Q-Exactive, veterinary drug residues*

Acknowledgement: *Authors thank Nuria Cortés and Josep Caixach of Organic Pollutants-Mass Spectrometry laboratory, CSIC, Spain. P.Kumar thanks Prof.Juliane Hollender of Swiss Federal Institute of Aquatic Science and Technology (EAWAG) for non target analysis.*

S-49
A NEW ALTERNATIVE FOR PERFORMING CONFIRMATORY ANALYSIS OF ANABOLIC STEROIDS IN MEAT BY MEANS OF HIGH RESOLUTION ACCURATE MASS MS AND MS/MS DATA

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A prominent trend which has been observed in recent years in the analysis of veterinary drugs and growth-promoting agents is the shift from target-oriented procedures, mainly based on liquid chromatography coupled to triple-quadrupole mass spectrometry (LC–QqQ–MS), towards accurate mass full scan MS (such as time of flight (ToF) and Fourier Transform (FT) MS). In a previous study the applicability of HR–Orbitrap–MS for confirmatory analysis of growth-promoting agents in meat was compared to that of a QqQ–MS. Validation according to CD 2002/657/EC demonstrated that steroid analysis based on single stage Orbitrap mass spectrometry, operating at a resolution of 50,000 FWHM, is indeed capable to compete with QqQ–MS in terms of selectivity/specificity, while providing excellent linearity (for most compounds > 0.99) but somewhat inferior sensitivity. Therefore our goal was to investigate the capability of a TripleTOF™(QqTOF) instrument to provide sensitive high resolution accura te mass MS data (> 25,000 FWHM) but at the same time MS/MS data at high acquisitions speeds. For the development of the detection method the fast Information Dependent Acquisition (IDA) combining a TOF–MS survey scan followed by dependent TOF–MS/MS scans will be compared to the more time consuming workflow of optimizing the collision energy per compound. Afterwards a validation according to the CD/2002/657/EC will be performed, in this way the accurate mass MS data is used to accurately quantify the targeted steroids and the additionally collected MS/MS spectra are used to confidently identify the compounds. The benefit of this instrument compared to the QqQ–MS is the ability to mine the data retrospectively for identifying non-targeted and unexpected compounds, which is beneficiary in the light of the national control plan of growth-promoting agents. When compared to the single stage Orbitrap, a lower resolution is obtained, however due to the acquired MS/MS data the Triple TOF™ is expected to provide an advantage especially for those compounds (i.e. some estrogens) for which the threshold of 20% of repeatability and intra-laboratory reproducibility, correlated to an inferior precision, was exceeded with the single stage Orbitrap. Finally, a thorough comparison and conclusion will be provided for the detection of anabolic steroids in meat by means of the TripleTOF™-MS in terms of linearity, precision, sensitivity and other relevant performance characteristics, as the validation is in progress.

Keywords: *TripleToF–MS; Orbitrap–MS; Anabolic steroids; meat*

S-50
MULTI-RESIDUE ANALYSIS OF VETERINARY DRUGS IN SHRIMP BY USING LC–MS/MS

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We describe the development and validation of a simple multi-residue LC–MS/MS method to determine 29 veterinary drugs including fluoroquinolones (enoxacin, norfloxacin, levofloxacin, ofloxacin, ciprofloxacin, lomefloxacin, danofloxacin, enrofloxacin, orbifloxacin, sarafloxacin, sparafloxacin, difloxacin, oxolinic acid, nalidixic acid, flumequine, and perfloxacin), sulfonamides (sulfamerazine, sulfamonomethoxine, sulfapyridine, sulfaquinoxaline, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfadimethoxine, and trimethoprim), and nitroimidazoles (dimetridazole, metronidazole, ipronidazole, and ronidazole) in shrimp. Samples are extracted with acetonitrile followed by a clean-up of the remaining fat with hexane by liquid-liquid extraction technique. The determination is achieved by liquid chromatography coupled to tandem mass spectrometry under positive electrospray mode. Mean recoveries of all compounds from the fortified sample are in the range from 80–115% with coefficients of variation lower than 10%. The limit of detections (LODs) are in the range from 0.1–1.0 µg/kg while the limit of quantifications (LOQ) are 0.3–5.0 µg/kg depending on the various types of veterinary drugs.

Keywords: *Multi-residue method, LC–MS/MS, veterinary drugs, validation*

S-51
DEVELOPMENT OF A MULTIRESIDUE MULTICLASS METHOD FOR THE DETERMINATION OF ANTIBIOTICS IN ENVIRONMENTAL AQUACULTURE MATRICES

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Nowadays, aquaculture is an emergent food industry representing a sector that contributes with almost half the fish consumed around the world. One of the main constraints of this practice is related with bacterial diseases responsible for serious economical losses. Treatment and prevention are usually conducted through the administration of antibiotics as medicated feed or by adding the drugs directly into the water (bath treatments). Apart from being used to ensure animal welfare, antibiotics can also be applied in farmed fish to stimulate growth, leading to their presence in the aquatic environment. It is also important to bare in mind that pharmaceuticals used in human and veterinary health can be accumulated in the surrounding ecosystems. For consumers, fish obtained from aquaculture may present such residues, which can be responsible for toxic effects and/or allergic reactions in individuals with hypersensitivity, and also can lead to the development of resistant strains of bacteria. The main purpose of this work was to develop a new analytical method for the assessment of a wide range of antibiotics in environmental matrices related with aquaculture, more specifically sediments and water originating from the Mondego estuary. After testing several organic solvents, a simple and effective extraction procedure was developed, based on a liquid-liquid acetonitrile extraction. Detection was performed with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS), allowing higher resolution and sensitivity and thus the advantage of reduction of the time required for analysis. The presented method is suitable to simultaneously determine the presence of antibiotics from 7 families in sediments and water: sulfonamides, tetracyclines, macrolides, quinolones, chloramphenicol, penicillins, and trimethoprim. Despite of the lack of specific legislation concerning environmental matrices, the method was validated to be applied in routine analysis based on the evaluation of the following parameters: specificity, recovery, precision, linearity and limits of detection and quantification. The method developed aims to be used as a routine monitoring tool for the presence of antibiotics in aquaculture surrounding environments.

Keywords: *Antibiotics; multi-detection; UPLC–MS/MS; aquaculture; sediments*

Acknowledgement: *This work was funded by FEDER through the Operational Program for Competitiveness Factors – COMPETE, and through FCT – Portuguese Foundation for Science and Technology under the project PTDC/AGR-ALI/122119/2010*

S-52
BIOMARKER DISCOVERY FOR ANABOLIC TREATMENT IN BEEF CATTLE: A META-ANALYSIS OF TRANSCRIPTOMIC DATA

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Introduction. Anabolic steroids are used in beef cattle to increase growth performances, despite their use is banned in the EU for the potential risk associated with residues in meat products. Indirect biomarkers have been often proposed to detect animals illegally treated but despite many advantages, they are often influenced by intrinsic or extrinsic factors (e.g. age, breed, diet). Meta-analysis of transcriptomic data by including a much broader dataset could allow a more reliable identification of gene expression biomarkers for samples classification (treated or negative).
Material and methods. Microarray data from 3 different batches of bovine skeletal muscle samples were analyzed for a total of 101 samples. The first batch includes samples from male Holstein cows, 12–25 months old experimentally treated with combinations of sexual steroids and corticosteroids (45 animals). The second one embraces muscle samples from male Holstein calves, 6–7 months old, experimentally treated with different combination of growth promoters (40 animals). The third one is composed by muscle samples from male mixed breed (Charolaise X Limousine) cows, 18 months old (16 animals). Data from batch 1 and 2 were used for training, whereas data from batch 3 were used for test. After quality control and filtering, data from batch 1 and 2 were corrected for batch effect using ComBat and normalized using quantile normalization. A support vector machines classifier was then trained in a bootstrap framework, to detect a reproducible transcriptional signature able to classif y new samples as treated or not and to provide a realistic estimate of performance on independent samples.
Results. Statistical analyses of microarray data from batch 1 and 2 allowed to identify a classifier, consisting in a set of 79 potential biomarkers, that correctly discriminated all treated and control animals in both batches. This classifier was then tested on batch 3, independently preprocessed and normalized, and confirmed a high classification accuracy (Matthews correlation coefficient equals to 80%). Conclusions. This study showed that a meta-analysis approach, developed combining bovine skeletal muscle gene expression signatures from different experiments with bioinformatic tools, enables to discover robust biomarkers for the identification of animals treated with anabolic steroids, despite individual variation and specific growth promoting treatment.

Keywords: *Anabolic steroids, cattle, biomarkers, meta-analysis, transcriptomic data*

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S-53
USE OF HIGH RESOLUTION MASS SPECTROMETRY IN FOOD SAFETY: IDENTIFICATION AND CONFIRMATION CRITERIA

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Mass spectrometry is a field that has suffered a rapid development in the recent years. Different technologies are constantly being developed, e.g. Triple quadrupole (QqQ), Ion trap (IT), Time of flight (TOF), Orbitrap and hybrid instruments such as q-TOF, q-Orbitrap, etc. Nowadays a wide range of instruments are available for food safety control laboratories and the current legislation is not yet updated to the novelties available in the field. When Decision 657/2002/CE was published, HRMS was basically applied in official routine analysis, mainly for the analysis of dioxins, using magnetic field instruments; TOF instruments were mainly used for research purposes. In the 2002–2010 period some scientific articles dealing with the subject were published, and helped to fill the gap. More recently, legal documents have introduced the new HRMS concepts, identification and confirmation criteria: SANCO 12495/2011 for pesticide analysis and CODEX Alimentarius Draft (May 2012) for Veterinary Drug Residues Analysis, but still some aspects to assess full confirmation have to be clarified. So far, triple quadrupole mass spectrometers have been the main instrumentation of analytical laboratories with high work load. In the Laboratory of Public Health Agency in Barcelona (LASPB), official control analysis using QqQ instruments includes the following criteria: chromatographic peak in both quantification and confirmation transitions, retention time identification, ion ratio confirmation within the permitted percentages of variation. However, some difficulties confirming results have been arising: coeluting interferences, isobaric compounds, etc. High resolution mass spectrometry (HRMS) is a powerful tool to overcome such difficulties. Common strategies for identification and confirmation using HRMS include: retention time permitted variation, molecular/precursor and product ion accurate mass, ion ratio between precursor and product ion, minimum experimental resolution. LASPB has established criteria for the need of HRMS confirmation of presence of contaminants in samples of animal origin: - Non compliant ion ratios of analytes – Presence of forbidden substances around CCα levels. – Absence of one transition in QqQ. – Presence of both transitions with retention time shift. – No fragmentation of some molecules Examples of the first cases are presented in this communication: Ronidazol analysis in muscle of different animal species and Hormone analysis in urine. For Ronidazol doubtful confirmation using QqQ was observed because mass fragments were not abundant enough and being classified as forbidden substance (96/23/CE Directive). Due to matrix interferences no suitable confirmation using QqQ for some hormones could be established. It has been shown that HRMS provides incomparable confirmatory performance with excellent quantitative capabilities to be used when obtaining doubtful results working with QqQ.

Keywords: *HRMS, LRMS, Food Safety, Confirmation criteria*

S-54
DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE LC–MS/MS ANALYSIS OF 14 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN MEAT AND MILK

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used substances in veterinary medicine for treating fever, pain and inflammation (e.g. coliform mastitis) in food-producing animals. Their widespread administration and occurrence in animal-derived products (meat and milk), impose a potential risk for humans. Maximum residue limits (MRLs) have been set for some (Commission Regulation 37/2010/EEC and amendments), implying the need for sensitive analytical methods to detect/identify/quantify possible residues.
This study aimed at developing and validating a multi-residue method for milk as well as for meat to simultaneously detect, identify and quantify 14 NSAIDs by liquid chromatography-tandem mass spectrometry (LC–MS/MS): carprofen, diclofenac, phenylbutazone, flufenamic acid, flunixin (or metabolite 5-hydroxy-flunixin in milk), ketoprofen, 4-methylaminoantipyrine (marker of metamizole), meloxicam, tolfenamic acid, mefenamic acid, niflуminic acid, naproxen, ramifenazone and salicylic acid (metabolite of acetylsalicylic acid).
Mass spectrometric parameters and chromatographic conditions were optimized on a Quattro Ultima Pt triple quadrupole (Waters) and the Acquity UPLC (Waters) system, respectively. H_2O/MeCN (95/5) + 0.1 % formic acid (solvent A) and MeCN +0.1 % formic acid (solvent B) were used for chromatographic separation. Extraction was performed using MeCN for both milk and bovine meat. The methods were validated according to Commission Decision 2002/657/EC considering performance characteristics such as specificity, linearity (R^2), recovery (RA), repeatability (RSDr), intra-laboratory reproducibility (RSDR), decision limit (CCα) and detection capability (CCβ).
The criteria of linearity ($R^2 \geq 0.99$) and specificity were fulfilled for all compounds and matrices studied. As for the other validation results, RA varied between 91% and 111% for meat, and between 89% and 107% for milk. RSDr and RSDR were found to be adequate for the different compounds (<11% and <18% for meat, and <15% and <21% for milk, respectively). The CCα and CCβ values were in the range of 0.5 to 642 µg/kg for bovine meat, whereas these were in the range of 0.13 to 55 µg/kg for milk.
The methods developed are suitable for identifying and quantifying NSAIDs in bovine meat and milk samples in view of food safety.

Keywords: *NSAIDs, meat, milk, LC-MS/MS*

S-55
DEVELOPMENT OF A MULTIRESIDUE MULTICLASS METHOD FOR THE DETERMINATION OF ANTIBIOTICS IN ENVIRONMENTAL AQUACULTURE MATRICES

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Nowadays, aquaculture is an emergent food industry representing a sector that contributes with almost half the fish consumed around the world. One of the main constraints of this practice is related with bacterial diseases responsible for serious economical losses. Treatment and prevention are usually conducted through the administration of antibiotics as medicated feed or by adding the drugs directly into the water (bath treatments). Apart from being used to ensure animal welfare, antibiotics can also be applied in farmed fish to stimulate growth, leading to their presence in the aquatic environment. It is also important to bare in mind that pharmaceuticals used in human and veterinary health can be accumulated in the surrounding ecosystems. For consumers, fish obtained from aquaculture may present such residues, which can be responsible for toxic effects and/or allergic reactions in individuals with hypersensitivity, and also can lead to the development of resistant strains of bacteria. The main purpose of this work was to develop a new analytical method for the assessment of a wide range of antibiotics in environmental matrices related with aquaculture, more specifically sediments and water originating from the Mondego estuary. After testing several organic solvents, a simple and effective extraction procedure was developed, based on a liquid-liquid acetonitrile extraction. Detection was performed with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS), allowing higher resolution and sensitivity and thus the advantage of reduction of the time required for analysis. The presented method is suitable to simultaneously determine the presence of antibiotics from 7 families in sediments and water: sulfonamides, tetracyclines, macrolides, quinolones, chloramphenicol, penicillins, and trimethoprim. Despite of the lack of specific legislation concerning environmental matrices, the method was validated to be applied in routine analysis based on the evaluation of the following parameters: specificity, recovery, precision, linearity and limits of detection and quantification. The method developed aims to be used as a routine monitoring tool for the presence of antibiotics in aquaculture surrounding environments.

Keywords: *Antibiotics; multi-detection; UPLC–MS/MS; aquaculture; sediments*

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S-56
ORGANIZATION AND PRELIMINARY RESULTS OF A PROFICIENCY TEST FOR PREDNISOLONE, CORTISOL AND PREDNISOLONE METABOLITES IN PORCINE URINE

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Prednisolone is a synthetic glucocorticoid whose use for growth-promotion in food-producing animals is in Europe. However, several cases of detection of residues of prednisolone in low concentrations in urine of pigs were observed over the last year in the framework of the Belgian control plan. The EURL (RIKILT, Netherlands) had proposed a threshold of 5 ng/mL for future control in both bovine and porcine urines. To confirm this threshold level, quantitative data have to be collected through member states. Biomarkers could also be used to distinguish between exogenous and endogenous prednisolone residues; even if this approach is still under study, prednisolone metabolites are potential biomarkers. The aim of this proficiency test was therefore to evaluate available quantitative confirmatory method for prednisolone and cortisol in porcine urine, as well as the capacity of participating laboratories to analyze prednisolone metabolites within the same methods. The PT materials were 4 lyophilized porcine urines, obtained from an animal experiment performed in the animal facilities of CER Groupe. Briefly, sows were treated either with prednisolone or tetracosactide hexaacetate (synthetic analogue of ACTH) and naturally voided urine were collected. Samples were sent to participants together with instructions, acknowledgment of receipt, results reporting and method description forms. Participants were informed that each sample could potentially contain prednisolone, cortisol, prednisone, 20α-hydroxyprednisolone and 20β-hydroxyprednisolone. If participation was mandatory for Belgian Official Control laboratories (2 labs), an invitation was also send to members of the EURL network and 3 official labs from other Member States have participated to the proficiency test. Homogeneity and stability studies were performed for each sample. Results as well as analytical methods of all participants will be described.

Keywords: *Prednisolone, cortisol, porcine urine, Proficiency Test*

S-57
FAST MULTIRESIDUE AND MULTI-CLASS QUALITATIVE SCREENING FOR VETERINARY DRUGS IN DIFFERENT FOODS OF ANIMAL ORIGIN BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY

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Use of veterinary drugs may result in the presence of drug residues in foods of animal origin such as milk, eggs, honey, and meat and constitutes a potential health risk for consumers.
Monitoring foods to ensure that drug residues are below the maximum residue limits (regulation (EC) N° 37/2010) can be made more efficient through the development of methods for detecting multiple drug residues of multiple classes. Although there exist, for edible tissues, published multi-class detection methods based on liquid chromatography coupled to tandem mass spectrometry, these methods involve a purification step such as solid-phase extraction, liquid-liquid clean-up microfiltration or a QuEChERS procedure.
Multi-class ultra-high-performance liquid chromatography combined with tandem mass spectrometry was developed for the analysis of more than 180 regulated or banned compounds of various classes (anthelmintics including benzimidazoles, avermectins, flukicides and others, antibiotics including amphenicols, beta-lactams, macrolides, pyrimidines, quinolones, sulfonamides, and tetracyclines, beta-agonists, corticosteroids, ionophores, nitroimidazoles, non-steroidal anti-inflammatory agents, steroids and tranquillizers).
Samples (meat, milk, egg and honey) are extracted with acetonitrile, without any additional purification step, and analysed by UHPLC–MS/MS.
The validation procedure was conducted according to European Union requirements (decision 2002/657/EC) for qualitative methods and covered detection capability (CCβ), selectivity, specificity, and stability. The method allows detection of at least 180 different drugs and has a false-compliant rate (β-error) of 5% at half the maximum residue limits or other maximum levels established under European legislation. In most cases, target value was set at 5 µg kg^{-1} for unauthorized compounds
At least one transition per compound was monitored. For some compounds, only one transition could be recorded because of the poor intensity and insufficient specificity of the second transition. With our method, all veterinary drugs were separated by 1.4 to 11 min in the positive mode and by 2.3 to 10.6 min in the negative mode.
The developed method has been used successfully for a surveillance check for the presence of these residues in meat including suspect samples (injection site) for the past 3 years. This method was also applied more recently to screen egg, honey and milk for veterinary drugs residues.

Keywords: *Multi residues, egg, honey, milk, muscle*

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S-58
PRELIMINARY STUDY ON THE PRESENCE OF PREDNISOLONE IN PORCINE URINE AND LIVER – HOW TO DISTINGUISH ENDOGENOUS FROM THERAPEUTICALLY ADMINISTERED PREDNISOLONE

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In Europe, hormones and corticosteroids are not authorized for use in food-producing animals as growth-promoting agents. However, prednisolone residues have recently been found in porcine urine samples collected at slaughterhouses. The aim of this work was therefore to look for prednisolone in porcine urine and liver, to determine if detected residues might be of endogenous origin and to check the possible relation with stress.
An in-house-developed analytical method was validated, combining immunoaffinity-based purification and Ultra-High Performance Liquid Chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). This method was applied to urine and liver samples collected from sows experimentally treated either with prednisolone or tetracosactide hexaacetate (synthetic analogue of ACTH).
Thanks to the performance of the analytical method, both cortisol and prednisolone were detected in all pig urine samples collected before or after administration of prednisolone or tetracosactide hexaacetate. High levels of prednisolone were found in porcine urine just after prednisolone administration, decreasing quickly to within the range detected in non-treated animals. In urine, the cortisol level varies depended on the time lapse between administration and sampling. On the other hand, prednisolone was detected also in liver samples of treated pigs. In this matrix, the cortisol level remained constant and prednisolone/cortisol level could be used to detect prednisolone administration at least 4 days after injection.
In conclusion, the best indicator for detecting illicit prednisolone administration to pigs seems to be the prednisolone/cortisol ratio in liver samples. This preliminary work must be confirmed by a larger-scale study and metabolites should also be included.

Keywords: *Prednisolone, cortisol, porcine urine, immunoaffinity column, UHPLC–MS/MS*

S-59
USE OF VETERINARY MEDICINAL PREMIXES UNDER RULES OF THE ANTIBIOTIC POLICY

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Antimicrobial resistance is a serious European and global problem encompassing both human and veterinary medicine, farm animal breeding, agriculture, environment as well as food and livestock trade. Foodstuffs and direct contact with animals are now proven means of antimicrobial resistance transfer from animals to man.
Because of the resistance increase of micro-organisms complicating the treatment of people, the antibiotic use in farm animals especially in a form of medicated feeds gives rise to a whole number of controversial opinions, since sub-therapeutic use of antimicrobial drugs in animals, as it may occur with a mass administration of medicinal products, enhances the resistance development. Now some countries of the European Union are already considering a prohibition of antimicrobial medicines administration in that pharmaceutical form to food animals particularly to pigs and poultry as those are the most frequent farm animal species in which the mass administration of medicines is used. Last year during a November session the European Commission presented on occasion of "the European Antibiotic Awareness Day, November 2012" so called "European Strategic Action Plan on Antibiotic Resistance 2011–2016"
Objectives of the study have been as follows:

- to compile all new provisions related to antibiotic policy, new strategic plans against antimicrobial resistance development and new regulatory measures for the use of medicated feeds in food animals as well as to show risks connected with the mass administration of antibiotics to animals;
- to draw attention to the situation in the area of antibiotic resistance and possible procedures to solve the problem (including the use of medicated feeds) in different EU countries and in the countries outside of EU as well as to the approach of concerned organisations such as OIE, EFSA and EMA, SANCO, WHO.
- to chart the situation in the Czech Republic –with respect to antibiotic consumption (focusing on premixes), the level of antimicrobial resistance to antibiotics, which occur most frequently in premixes for feed medication and also aim at supervision in area of medicated feeds.

The safety of feed mixtures, as we are trying to show in the study, is necessary to assess especially from view of the risks for animal health (and subsequently of people), that are caused by campaign manufacturing of medicated and non-medicated feed mixtures by using a common mixing equipment. During manufacturing process there arises risk of cross-contamination of feeds by antibiotics. When using medicated feeds in practice, we can often encounter an inadequate dosing of medicated feed and consequently also its active substance or other inappropriate therapeutic use. Both contribute to the selection pressure increase on bacteria and to the antimicrobial resistance increase.
However the antibiotic medicated feeds represent only a partial part of the global problem that is called antimicrobial resistance and it is impossible to solve it separately from the remaining parts involved. Further discussions on adjustment veterinary legislation should continue and the requirement for inclusion of medicated feeds in veterinary medicinal products is certainly legitimate. But simultaneously there is necessary a cooperation of all parts concerned not just in the veterinary area. Although executive authorities can provide regulatory framework for the problem solving but crucial is especially the responsible approach of veterinarians and animal breeders towards the antibiotic use.
We regard as more than useful to prepare for veterinarians and breeders (above all pigs and poultry) technical material explaining in details the recommended procedures for antibiotic use. Such a guideline should include as the correct procedures for infectious diseases diagnostics as the way of suitably conducting treatment by use of antibiotics – with regard to individual indications, dosing and possible combinations of antimicrobial medicinal products (the unsuitable combinations of individual antimicrobial substances should be particularly emphasized).

Keywords: *Antibiotics, medicines, premixes*

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LM-1
A SURVEY STUDY ABOUT THE AWARENESS LEVEL OF TURKISH CONSUMERS IN REGARD TO DEPENDABILITY OF THE FOOD PACKAGING MATERIALS

Beraat Özçelik[1], Ümit Altuntaş[2], Selin Hande Başaran[3], Sandra Yüzarı[4]

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In the production of packaging materials such as plastic, glass, paper etc., some low molecular weighted compounds such plasticizers, surfactants are incorporated to improve characteristics of these materials. Integrated compounds can be migrated from packaging materials to foods depending on temperature, time and food composition. Because of the exposure of consumers to these compounds chronically, hazardous health problems can be occurred. These hazardous migrated materials for consumer health and their effects have been discussed in the literature up to now. The aim of this study is to present consumers' attitude and behavior related to packaging materials and to measure their awareness. Studies on packaging materials and their migration into foods are investigated; common materials used for packaging and their properties, area of use, migrated constituents and factors affecting migration were determined. Then a questionnaire is prepared to survey awareness of the Turkish consumers with regard to dependability of packaging materials and consumer awareness on this issue was evaluated by using obtained results. Responses of the consumers to the asked questions were statistically analyzed to determine whether there is difference based on demographic properties (sex, age, educational level, etc.) of consumers. The questionnaire ~~is~~ was mainly conducted on 21-30 years old people, university students and people living in İstanbul. One of the most important data as a result of the questionnaire study showed that consumers was not aware about migration of toxic substances from package although they seem informed in some questions. Participants frequently did not know about the reason of their responses or behaved toward their habituation. Therefore, raising awareness of community about that prevalent issue is a necessity for researchers. As a result of study, although half of the consumers had information about migration of foods, they also applied to some hazardous preferences.

Keywords: *Bisphenol A, packaging material, migration, consumer awareness*

LM-2
DETERMINATION OF BENZOPHENONE AND DERIVATIVES MIGRATING FROM PAPER AND CARDBOARD FOOD PACKAGINGS BY GC-MS

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The qualitative and quantitative analyses of benzophenone and other 17 derivatives were performed by GC-MS system which is equipped with a gas chromatography (Agilent Technologies 7890A), mass spectroscopy (Agilent Technologies 5975C), an auto-sampler (Agilent Technologies 7693A). In this study, 11 different paper or cardboard materials (7 recycled and 4 virgin) were analyzed for their benzophenon and derivatives of benzophenon contents. Seventeen benzophenon derivatives (Benzophenone, 2-methylbenzophenone, 1-Hydrocyclohexyl phenyl ketone, N-ethyl-p-toluene sulphonamide, 2-Hydroxybenzophenone, 3-Methylbenzophenone, 4-Methylbenzophenone, 2,2-Dimethoxy-2-phenylacetophenone, Methyl-2-benzoylbenzoate, 4-Fluoro-4-Hydroxybenzophenone (IS), 4-Hydroxybenzophone, Flavone (IS)2-Ethylhexyl-4-(dimethylamino) benzophenone, 2-Methyl-4-(methylthio)-2-morpholinoprophenone, 4-ısopropylthioxanthone, 2-Isopropylthioxanthone, 4-benzoylbiphenyl, 2,2-Diethyl-9H-thioxanthen-9-one, 4,4-Bis(diethylamino)benzophenone) were investigated in the samples. The method was evaluated by validation parameters such as calibration range, limit of detection, limit of quantification, repeatability, accuracy and precision, and recovery. The maximum values by specific Migration Test (40c 10d TENAX Sımulant) was 10.83 ppm for benzophenon , 0.53 ppm for 1-Hydrocyclohexyl phenyl ketone, 0,47 ppm for 4-Methylbenzophenone, 0.22 ppm for 2,2-Dimethoxy-2-phenylacetophenone, 0.4 ppm for Methyl-2-benzoylbenzoate. The other derivatives investigated in the samples were not able to be detected in the samples.

Keywords: *Benzophenon, derivatives, paper and cardboard, packaging, migration*

Acknowledgement: *Dikran Acemyan the Owner of Duran Dogan Printing and Packaging Inc.*

LM-3
QUECHERS METHOD FOR THE DETERMINATION OF ORGANOPHOSPHORUS, ORGANOCHLORINE AND PYRETROID PESTICIDES RESIDUES IN VEGETABLES.

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Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe, the QuEChERS method is based on work done and published by Anastassiades et al (1). This technique offers a user-friendly alternative to traditional liquid-liquid and solid phase extractions. The QuEChERS method employing acetonitrile or ethyl acetate for the extraction was harmonized for the determination of organophosphorus, organochlorine and pyretroid pesticides residues in tomates and onion.
The method for the organochlorine and pyretroid pesticides residues involves the extraction of the sample with acetonitrile containing 1% acetic acid and simultaneous liquid-liquid partitioning formed by adding anhydrous magnesium sulfate plus sodium acetate, followed by a cleanup in dispersive solid-phase extraction with primary secondary amine (PSA). The method for the organophosphorus pesticides residues involves the extraction of the sample with ethyl acetate in the presence of anhydrous sodium sulfate, after removal of residual water are performed dispersive solid-phase extraction with PSA and anhydrous magnesium sulfate.
The fortification levels were selected according to National Maximum Residue Level or the corresponding Codex standard and the pesticides residues extracted were analyzed by gas chromatography (GC–NPD, GC–ECD).
Sampling of tomates and onions were carried out in 5 farmer and pesticides residues analised by QuEChERS method. The samples did not detect the presence of pesticides.
This work was carried out under the project: **"**Harmonizing and validation of analytics methods **f**or the vigilance of risk for human health of residues and chemical contaminating presents in food".

[1] M.Anastassiade, S.J. Lehotay, D.Stajnbaher, F.J.Schenck, J.AOAC International 86, p.412-431 (2003).
[2] INISAV. PT-QM-LR-03 Validación de métodos de ensayos de residuos de plaguicidas (2010).

LM-4
RESIDUES OF QAC IN FOOD OF ANIMAL ORIGIN – OVERVIEW OF FINDINGS BY CVUA FREIBURG IN 2012/13

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Quaternary ammonium compounds (QAC) are used as biocides in processing of food of animal origin. They are surface active and due to their long alkyl chains also fat soluble. Residues are therefore expected in processed food of animal origin, if applied. In 2012 unexpected findings in food of plant origin brought this substances into focus [1,2].
The EURL AO has established an analytical method in 2012 to check residues of Benzalkonium chloride (BAC) and Didecyldimethylammonium chloride (DDAC) in food of animal origin. The method has been tested with processed meat, milk and dairy products in a method performance assessment RELANA® [3] in 2012 with satisfactory results.
In 2012 and 2013 processed food of animal origin was tested for residues of QAC. Main findings were in hard cheese, dairy products, liquid whole egg and ice cream. Residues above the LOQ were also observed in nearly 50 % of baby food products.
The Standing Committee of the Food Chain and Animal Health (SCoFCAH) has established a provisional MRL for BAC and DDAC of 0.5 mg/kg product according to Annex I of Regulation (EC) No 396/2005 [4,5]. According to German authorities residues in processed food have to be recalculated on the raw product (e.g. cheese → milk). Additional entry of QAC during food processing is not taken into account.

[1] Friedle et al, Determination of Quaternary Ammonium Compounds (QAC) in Food Products, Poster Presentation, EPRW 2012 Vienna
[2] Schüle et al, Residues of Quaternary Ammonium Compounds (QAC) in Fruits and Vegetables,Poster Presentation, EPRW 2012 Vienna
[3] Method Performance Assessment "Quaternary Ammonium Compounds (QAC) in vegetable (banana, basil) and animal products (milk quark, meat) Relana® by Lach&Bruhns Partnerschaft, Hamburg
[4] Guidelines as regards measures to be taken as regards the presence of Benzalkonium Chloride (BAC) in or on food and feed agreed by the Standing Committee of the Food Chain and Animal Health (SCoFCAH) on 13 July 2012 and modified on 5 October 2012
[5] Guidelines as regards measures to be taken as regards the presence of Didecyldimethylammonium chloride(DDAC) in or on food and feed agreed by the Standing Committee of the Food Chain and Animal Health (SCoFCAH) on 13 July 2012 and modified on 5 October 2012

LM-5
MULTIMYCOTOXINS ANALYSIS USING A NEW SOLID PHASE EXTRACTION SORBENT BASED ON MOLECULARLY IMPRINTED POLYMERS

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Clean-up and sample preparation before analyses are key steps in mycotoxins analysis particularly for their detections at a trace level (range of µg/kg). Solid phase extraction (SPE) based on Molecularly Imprinted Polymer (MIP) sorbents is a method of choice to reach these levels. MIP is a synthetic polymer material that mimics antibodies recognition properties and that binds a target molecule or multiple targets by combining MIP sorbents. MIP has the advantages to be chemically and thermally stable, compatible with all solvents and cost-effective. These polymers are used as a powerful method for clean-up and pre concentration applications. The efficiency of a method using MIP as selective sorbent for solid-phase extraction will be shown with respect to the clean-up and pre-concentration in different matrices. A highlight on the simultaneous analysis of several mycotoxins with a MIP based SPE clean-up will be displayed for cereal matrices (corn and wheat). An efficient multimycotoxins sample preparation method based on MIP is now available for different matrices and really suits for fast LC-MS/MS analysis. Aflatoxins, Ochratoxins A, Zearalenone, Fumonisines, T2 and HT2 mycotoxins have been quantified with good recoveries according to EU regulation.

Keywords: *Mycotoxins, MIP, SPE, clean up, LC MSMS*

LM-6
FORMATION OF PYRRALINE IN THE MAILLARD REACTION IN A SACCHARIDE–LYSINE MODEL SYSTEM

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Modern diets are largely heat-processed, as a result, high levels of advanced glycation end products (AGEs) are found in diets. It is generally accepted that dietary AGEs are contributed to increase oxidant stress and inflammation, which are linked to the recent epidemics of diabetes and cardiovascular disease. Pyrraline, an advanced glycation end product, was used as an AGEs marker to evaluate the formation of AGEs in diet foods. In the this study, model systems consisting of different saccharides and lysine were heated at different times, temperatures and initial molar ratios of saccharide to lysine under microwave heating conditions in order to investigate the formation of pyrraline. Different saccharides including fructose, glucose, lactose and sucrose in a 0.2 M phosphate buffer at pH 6.8 were employed. The initial molar ratio of saccharide to lysine varied from 0.125:1 to 8.000:1, heating times was from 5 to 60 min and heating temperatures ranged from 60 to 220°C. The pyrraline was detected and quantified by an electrospray ionization liquid chromatography–mass spectrometry. Increasing in initial molar ratios of saccharide to lysine can significant promote the formation of pyrraline. Among different saccharides used, lactose rendered the highest pyrraline (4.56 µg/mL), the pyrraline formation rate was influenced by the type of reducing saccharide involved in the reaction, the formation rate decreased in the following order: lactose > fructose > glucose > sucrose. Besides, different saccharides all reached the maximum formation of pyrraline at 140°C except sucrose. Nevertheless, heating times had no pronounced effect on formation of pyrraline except lactose used. In a word, the formation of pyrraline by saccharide–lysine model system is a dynamic reaction.

Keywords: *Advanced glycation end products (AGEs), Maillard reaction, pyrraline, saccharide–lysine model system*

LM-7
ANALYSIS OF FREE ADVANCED GLYCATION END PRODUCTS IN SOYBEAN SAUCES

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Advanced glycation end products (AGEs) were a series of highly oxidant compounds generated by Maillard reaction, and the intake of AGEs (especially AGE free adducts) had been considered to play a critical role in the progress of many chronic diseases. Detection of dietary AGEs is very critical with respect to food safety. In this study, high performance liquid chromatography-mass spectrometry (HPLC-MS) was used for the quantitative determination of free Nε-(carboxymethyl) lysine (CML, a major AGEs) in randomly selected 33 types of soybean sauces. The effects of soybean sauce dosage, eluent composition and eluent volume on determination of free CML was investigated. The following optimum pretreatment condition for free CML was obtained as follows: soybean sauce dosage of 0.5 mg amino acid nitrogen equivalent, eluent composition of methanol-water (1:9, v/v) and eluent volume of 5 mL. Free CML levels in light soybean sauce and dark soybean sauce were found to range from 224.6130 to 954.1564 μg/mL per sample and 612.3727 to 821.0182 μg/mL per sample, respectively. Therefore, the high content of free CML in soy sauces should be drawn more attention to for food technologists.

Keywords: *Advanced glycation end products (AGEs), Nε-(carboxymethyl)lysine (CML), Soybean sauce, Maillard reaction, Quantification*

LM-8
AUTOMATIC IDENTIFICATION OF KNOWN CHEMICAL RESIDUES AND CONTAMINANTS IN FOOD SAMPLES USING ACCURATE MASS LC-MS/MS SCREENING TECHNIQUES

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Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC–MS/MS using an AB SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode. Full scan MS and MS/MS data was explored to identify knowns using extensive XIC lists of target compounds. Analytes were identified with high confidence based on retention time matching, mass accuracy, isotopic pattern, MS/MS library searching and elemental formula calculation based on MS and MS/MS ions. It was found that the use of MS/MS information is crucial to minimize false positive results. The latest revision of TripleTOF® data processing software makes this procedure intuitive and fast.

Keywords: *Chemical Residues and Contaminants, Accurate Mass LC-MS/MS, Screening*

LM-9
EFFECTS OF SODIUM SULPHITE EXTRACTION MEDIUM ON THE AMINO ACID COMPOSITION OF SOME GOURD SEEDS PROTEIN ISOLATES

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Protein isolates of Citrullus colocynthis, Citrullus vulgaris, Lageneria siceraraia I (African Wine Kettle gourd), Lagenaria siceraria II (Basket Ball gourd) and Lageneria siceraria III (Bushel Giant Gourd) gourd melon seeds were produced using alkali extractions under two different extraction media of sodium hydroxide and sodium sulphite. Protein isolates produced with sodium sulphites had higher total amino acid content (TAA) (904.60 to 933.90) mg/g protein and essential amino acid content (EAA) (419.20 to 473.20) mg/g protein than those extracted with sodium hydroxide with (TAA) (815.70 to 876.50) mg/g protein and (EAA) (409.70 to 426.30) mg/g protein. Sodium sulphite increased the total sulphur amino acids in the protein isolates with values ranging from 35.40 to 40.60 mg/g protein compared with sodium hydroxide extracted protein isolates (30.80 to 37.50) mg/g protein respectively. Hence, increasing the anti-oxidant effects of the protein isolates produced due to the in creased sulphur amino acids produced with sodium sulphite. SDS–PAGE Gel electrophoresis of the protein isolates gave positive correlation between the types of amino acids under these two different extraction media.

Keywords: *Sulphure amino acid, Protein isolates, essential amino acid, sodium sulphite*

LM-10
AUTOMATIC IDENTIFICATION OF KNOWN CHEMICAL RESIDUES AND CONTAMINANTS IN FOOD SAMPLES USING ACCURATE MASS LC-MS/MS SCREENING TECHNIQUES

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Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC–MS/MS using an AB SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode. Full scan MS and MS/MS data was explored to identify knowns using extensive XIC lists of target compounds. Analytes were identified with high confidence based on retention time matching, mass accuracy, isotopic pattern, MS/MS library searching and elemental formula calculation based on MS and MS/MS ions. It was found that the use of MS/MS information is crucial to minimize false positive results. The latest revision of TripleTOF® data processing software makes this procedure intuitive and fast.

Keywords: *Chemical Residues and Contaminants, Accurate Mass LC-MS/MS, Screening*

LM-11
AUTOMATIC IDENTIFICATION OF UNKNOWN AND UNEXPECTED CHEMICAL RESIDUES AND CONTAMINANTS IN FOOD SAMPLES USING ACCURATE MASS LC-MS/MS SCREENING TECHNIQUES

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Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) is a powerful analytical tool for the analysis of polar, semi-volatile, and thermally labile compounds of a wide molecular weight range, such as pesticides, veterinary drugs, mycotoxins and other food residues. Mass analyzers based on triple quadrupole technology operated in Multiple Reaction Monitoring (MRM) mode deliver highly selective and sensitive quantitative results and are therefore well established for multi-target screening and quantitation of food contaminants. However, the use of triple quadrupole based mass analyzers is limited to targeted screening and quantitation. But there is an increasing demand for retrospective and non-targeted data analysis. High resolution and accurate mass instruments are capable of performing targeted and non-targeted screening in a single LC–MS/MS run. Here, a generic QuEChERS procedure was used to extract residues and contaminants from fruit and vegetable samples. Extracts were subsequently analyzed by LC-MS/MS using an AB SCIEX TripleTOF® system operated in high resolution accurate mass MS and MS/MS mode. Full scan MS and MS/MS data was explored to identify known-unknowns using non-targeted data processing tools. Sample-control-comparison was successfully used to find unexpected contaminants. Identification was based on MS and MS/MS information, including formula finding, ChemSpider searching, and automatic MS/MS fragment ion interpretation. This challenging data processing workflow was automated and allows easy result review and reporting in the latest revision of TripleTOF® software.

Keywords: *Unknown screening, identification, software, Time-of-flight, LC-MS/MS*

J-72
A NEW, PRECISE AND ACCURATE METHODOLOGY FOR RAPID DETERMINATION OF 12 MYCOTOXINS IN DIFFERENT FOOD COMMODITIES IN ONE RUN BY USING LC–MS/MS

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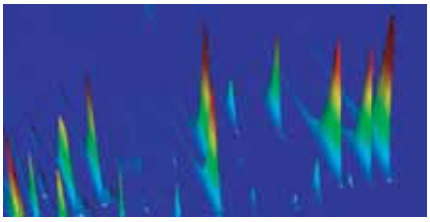
Background: Mycotoxins are carcinogenic substances produced by various fungi species growing on many agricultural commodities and processed food, either in the field or later during storage. The term mycotoxins appeared in the scientific literature since 1960 when 100 000 turkey poults died due to the ingestion of peanuts contaminated with secondary metabolites from Aspergillus Flavus. Commission Regulation (EC) No 1881/2006 and its amendments have set maximum residue levels (MRLs) for 13 mycotoxins, due to their toxic, carcinogenic, estrogenic, mutagenic, teratogenic, and immunotoxic effects on humans and animals. It is therefore necessary to apply very sensitive and reliable analytical methods able to reach the regulatory levels establish by the EC.
Aim of the study: The aim of our study has been to develop a simple and fast multi-mycotoxin method able to cover, in a single run, mycotoxins which are Aflatoxin B1, B2, G1, G2, Ochratoxin A, T-2 toxin, Deoxynivalenol, Nivalenol, Neosolaniol, Zearelanone, Sterigmatocystine, Fusarenone-X (aflatoxin M1 and patulin are developing as a separate methods) – all of them mentioned by the European Union (EU) food legislation.
Materials and methods: We have provided peanuts, pistachio, hazelnuts, chocolate nut paste, red pepper, dried fig, chips (containing maize and peanut) from the local markets. Mycotoxins have been extracted by the Jasem extraction method in 20 minutes, followed by liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (LC–ESI-MS/MS, Agilent's Triple Quad MS 6460). Run time takes only 12 minutes (run to run). Jasem extraction steps for peanuts are following: weigh 5.0 g fine blended peanut sample into a 50 ml polypropylene falcon - add 20 ml Reactive 1 onto sample – and vortex 15 minutes – than centrifuge at 2000 rpm for 5 minutes. Transfer 2 ml of supernatant into glass experiment tube and add 2 ml Reactive 2 onto sample and vortex. Transfer the bottom layer into a HPLC vial and inject into the system.
Results: We developed Jasem Multi-Mycotoxin LC–MS/MS method where there is no need for sample prep such as Liquid-Liquid extraction or any concentration steps.
High sample throughput: Our method's sensitivity is in the range of 0.06–2.5 µg/kg which is below the MRLs specified in Commission Regulation (EC) No 1881/2006 and amendments.
Conclusion: The Jasem new sample prep method, with reference to its goals, is consisting of only two simple extraction steps without any concentration or solid-phase extraction and it takes only 20 min. The Jasem method offers cost savings compared to existing methods and a very easy & fast sample preparation, excellent repeatability: Jasem's technology focuses on improvement of the sample preparation part, since this analysis-part is till now taking dis-proportional long time in existing reference & house methods.

Keywords: *Mycotoxin analysis, LC-MS/MS*

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